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EUTROPHICATION OF SURFACE WATERS - LAKE TAHOE: LABORATORY AND PILOT POND STUDIES

Lake Tahoe Area Council
South Lake Tahoe, California

May 1969

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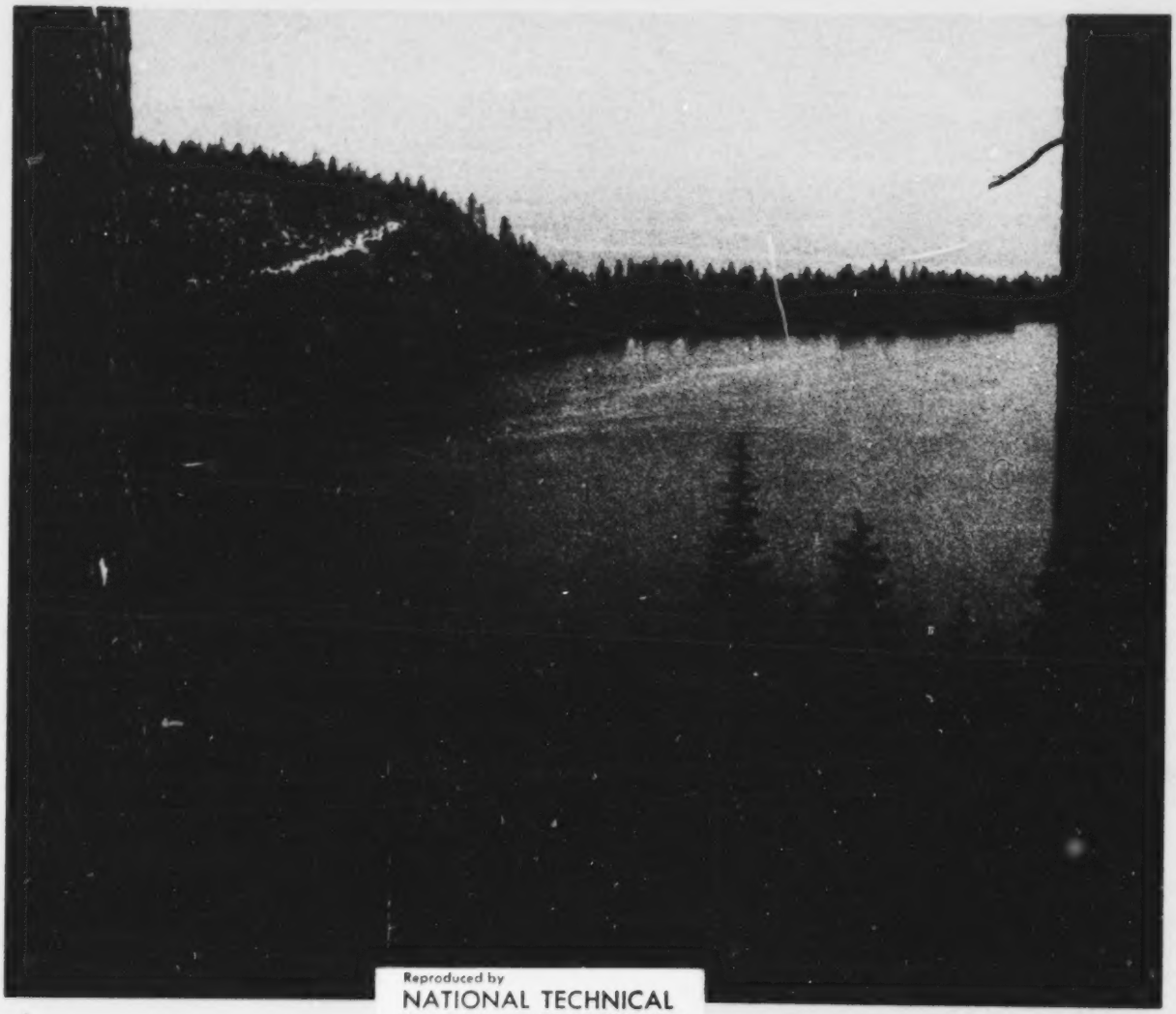
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EUTROPHICATION OF SURFACE WATERS—LAKE TAHOE

LABORATORY AND PILOT POND STUDIES

Second Progress Report
(FWPCA Grant No. WPD-48)
May 1969



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Lake Tahoe Area Council
South Lake Tahoe
California

SECOND PROGRESS REPORT

EUTROPHICATION OF SURFACE WATERS - LAKE TAHOE LABORATORY AND PILOT POND STUDIES

by

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FEDERAL WATER POLLUTION CONTROL ADMINISTRATION
U. S. Department of the Interior
FWPCA Grant No. WPD 48-02
Demonstration Project - Eutrophication of Surface Waters

May 1969

Lake Tahoe Area Council
South Lake Tahoe, California 95705

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PREFACE

This is the second in a series of reports on the progress of FWPCA Demonstration Grant No. WPD-48 to the Lake Tahoe Area Council for the purpose of identifying and evaluating the various sources of algal growth stimulants which may lead to the eutrophication of surface waters — with special reference to their implications to Lake Tahoe.

The First Progress Report, subtitled "Bioassay of Nutrient Sources" and dated May 1968, presented the results of bioassays of waste water effluents, surface runoff, and groundwater seepage for the presence of biostimulants and relative ability to advance eutrophication. It also compared batch and continuous culture techniques as tools for bioassay of waters. Two major findings emerged:

1. That there is an urgent need for the development of a standard bioassay technique capable of yielding replicable results interpretable in terms of field conditions.

2. That although laboratory bioassay methods are imprecise, they nevertheless reveal the presence of growth stimulants worthy of evaluating under Lake Tahoe conditions.

The first of these findings lent further support to the FWPCA's program to develop a standard bioassay procedure.

The second finding was the justification for the study herein reported, which is concerned both with a demonstration of the significance of laboratory bioassays to the eutrophication of Lake Tahoe and with further bioassay of nutrient sources in surface waters. The results reported are therefore pertinent to the fundamentals of bioassay as well as to the potential of various possible influents to increase the natural rate of eutrophication of Lake Tahoe.

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CHAPTER I

INTRODUCTION

NEED FOR STUDY

Need for the study herein reported derives from two major considerations: 1) a growing concern for the decline in quality of surface waters of the United States, and 2) the desire of millions of citizens to preserve the clarity of Lake Tahoe. The two differ only in order of magnitude of the associated problem known as eutrophication.

The nutrient-rich condition which characterizes eutrophic water is not always the result of man's activities upon the earth. Many streams, lakes, and ponds even under wilderness conditions support a rich flora and fauna, with cycles of growth which in some instances cover the water surface with a green scum of algae. In other situations, particularly in flowing streams, the nutrient concentration has remained low enough that the water is well suited to such high levels of use as domestic water supply while at the same time supporting a good fish fauna and the food chain on which it depends. In a few instances, of which Lake Tahoe is a notable example, the nutrient content has remained at such exceedingly low levels that the water quality is spectacular because of its nutrient-poor (oligotrophic) condition.

In all three of the foregoing natural situations the source of nutrients is the degradation of rocks and the decay of organic matter washed in from land surfaces and, generally, recycled within the water itself. Highly eutrophic waters are objectionable to man because the excess aquatic growth renders them aesthetically unattractive or otherwise unsuited to beneficial use without expensive treatment. Waters which support a wide spectrum of beneficial uses are pleasing to man. And his reaction to the clarity of the waters of Lake Tahoe is generally one of sheer delight.

As both the activities and the numbers of man have multiplied so has the percentage of the water resource which has eutrophic characteristics. Green scums, shoreline rocks enslimed with hairy filaments, and shallows clogged with weeds have appeared in waters formerly free of such nuisance. Algal blooms have aroused public indignation and increased the cost of obtaining pure water. To combat such loss in water quality, regulatory agencies have increasingly set forth water quality criteria and standards intended to preclude the discharge of growth stimulating factors into receiving waters.

But what are these growth stimulating materials; and what is their origin? The need for study is related to both aspects of this question.

The obvious source of nutrients is effluent discharged as municipal, agricultural, and industrial waste water. First attention to such wastes, however, was logically directed in the past to its oxygen demanding properties (BOD) and to their deleterious effect on aquatic life and on the aesthetic quality of water which various other beneficial uses require. Consequently, the art of sewage treatment developed around biostabilization of degradable organic matter and until quite recently treatment processes have become progressively more sophisticated only in their abilility to oxidize organic matter. Unfortunately, the oxidized forms of nitrogen and phosphorus are in themselves significant growth stimulants, and their presence in waste water, along with biosynthesized vitamins, amino acids, trace elements, and other growth factors found in biologically treated wastes, raises serious questions as to the suitability of conventional waste treatment for control of nutrients influencing eutrophication.

In response to these questions the concept of nutrient removal from sewage effluent has recently had widespread appeal in water quality management. Because a few of the blue-green algae, one of the most important representatives of nuisance algal blooms, can fix nitrogen from the atmosphere, and because of the increasing quantities of nitrogen in rain, it is widely suggested that phosphorus is the critical nutrient and should be the first to be removed from sewage effluents. Nitrogen and phosphorus removal has been advocated and is already being practiced in the South Lake Tahoe area.

There is, therefore, a need to determine several factors in relation to sewage effluents, including:

1. The concentrations at which nitrogen and phosphorus will trigger or support serious algal growth.

2. The algal growth stimulating effect of sewage from which nitrogen and phosphorus has been removed.

3. The ability of practical nutrient removal processes to reduce nitrogen and phosphorus to levels below that critical to algal growth.

The assumption that domestic and industrial waste water effluents are the principal source of nutrients is not necessarily valid. In many instances in the lake country of the middle west fertilizing of agricultural land and animal manure disposal practices are the critical factors in eutrophication of lakes. Consequently there is a need to evaluate the relative role of sewage effluents and other sources of nutrients in the stimulation of algal growth in surface waters.

With specific reference to Lake Tahoe, where the basin is forested, population is increasing, and land use encourages erosion and follows the same pattern as other urban developments, it is particularly important to evaluate the various sources of nutrients. Export of sewage effluents from the basin is well advanced both in practice and in planning for the future. Approximately 50 percent of the sewage generated within the Basin is now being exported from the watershed. Collection and transport systems to export the remainder are planned for completion by the end of 1970. Here the need for study takes on other aspects:

1. The residual ability of tertiary (nutrient stripped) sewage effluents to stimulate algal growth.

2. The ultimate fate of nutrients removed from sewage in the Tahoe Basin.

3. The overall amount of nutrients reaching Lake Tahoe annually from the normal processes of nature in the basin (including precipitation).

4. The effect of man's near-shore and shoreline modifications and activities on the biology and natural beauty.

5. The significance of the findings of laboratory assays in terms of Lake Tahoe water under lake conditions.

In relation to the foregoing needs it has been suggested that it would be particularly ironical and tragic if the nitrogen stripped from sewage found its way into the lake via rainfall and phosphate via pickup from landfill, while the purified water from which they were removed was needlessly exported from the basin. Although it has not been shown that such is the case, the speculation underscores the need for studies of the type herein reported.

Because shoreline development and construction may influence both the input of nutrients and the way the lake responds to them, there is a need to interpret the results of assays in relation to:

1. Development of flood plains, meadows, and marshlands.

2. Construction of marinas, lagoons, and breakwaters.

3. General construction practices throughout the watershed.

OBJECTIVES OF STUDY

The general objectives of the study are implicit in the need outlined in the preceding section. Specific objectives include:

1. To determine by the most effective laboratory bioassay techniques available whether there is present in effluents from waste water treatment processes, or in surface wash or groundwater seepage from inhabited or uninhabited areas, materials capable of stimulating algal growth in surface waters; and at what concentrations they may be significant.

2. To demonstrate by studies on artificial ponds the applicability to Lake Tahoe of the results of laboratory assays of possible inputs to the lake.

3. To evaluate the danger to Lake Tahoe of man's waste effluents and land practices in the basin, on the basis of results of studies in pilot-scale experimental ponds and a survey of the potential yields of various nutrient sources within the basin.

SCOPE OF REPORT

The report is concerned primarily with the first two of the three foregoing objectives. In relation to the first objective the study concentrated on systematic assays of raw sewage; primary, secondary, and tertiary effluents; and surface runoff from inhabited and uninhabited areas – all being sources shown in the First Progress Report [1] to be important to Lake Tahoe. Results of both flask and chemostat assays of such materials, and of Lake Tahoe water itself, are presented and evaluated in detail. However, little data directly related to the effect of shoreline development and construction are presented. More direct work on these problems is being planned for the future.

Relative to the second objective, results are presented on growth studies (assays) of algae in experimental ponds simulating the shallow areas of Lake Tahoe when sewage effluents are added. Preliminary experiments with creek water from inhabited and uninhabited areas of the Tahoe Basin are also discussed in the report. Finally, the results of flask, chemostat, and experimental pond bioassays are evaluated in terms of the eutrophication of surface waters and of Lake Tahoe in particular.

ORGANIZATION OF STUDY

The study was made possible by a Demonstration Grant (No. WPD-48) to the Lake Tahoe Area Council by the Federal Water Pollution Control Administration of the U. S. Department of the Interior. Financial responsibility of the project was maintained by the Council. Technical direction of the work was provided by Professor P. H. McGauhey, Dr. G. A. Rohlich, and Dr. E. A. Pearson of the Lake Tahoe Area Council Board of Consultants. In planning experiments and in evaluating the data the Board was assisted by limnological consultants: Dr. Arthur D. Hasler, University of Wisconsin, Dr. Charles E. Goldman, University of California, Davis, and sanitary engineer Dr. E. J. Middlebrooks, University of California, Berkeley. Experimental work was conducted by a resident staff including Dr. Milton Tunzi and Dr. D. B. Porcella, biologists, Mr. Aleti Adinarayana, sanitary engineer,

Mrs. Florence Kupka and Mr. Peter Cowan, laboratory technicians. Cooperating with the Lake Tahoe Area Council and its consultants in planning the demonstration program, assembling data, and developing facilities were such public agencies as: the University of California; California Regional Water Quality Control Board No. 6; Nevada State Department of Public Health; California State Department of Fish and Game; U. S. Public Health Service, Region IX; Federal Water Pollution Control Administration; California State Department of Public Health; California State Water Resources Control Board; Douglas County Health Department; and Placer County Health Department.

Laboratory facilities, outdoor space for pilot ponds, and facilities for staff were developed at Tahoe City, California, through the cooperation of the California Department of Fish and Game which maintains a hatchery building at that location. The U. S. Coast Guard and Placer County cooperated in the project by providing a site for a lake water pumping station and easement for a pipeline leading to the pilot ponds. Cooperation of the University of California at Davis and Berkeley helped minimize the cost of the laboratory experiments and lake sampling. The North Tahoe Public Utilities District; the South Tahoe Public Utilities District; and the Reno-Sparks Wastewater Treatment Works likewise cooperated in the sampling activities necessary to the project.

ACKNOWLEDGMENTS

The Lake Tahoe Area Council, its Board of Consultants, and the Demonstration Project staff acknowledge with thanks the cooperation and assistance provided by the many agencies and individuals who were helpful to the project during the past year.

CHAPTER II

THEORY AND RATIONALE

INTRODUCTION

In designing experiments suited to the objectives of the study outlined in Chapter I, consideration must be given to a number of fundamental questions and to the extent to which they must be answered in order that the objectives be achieved. For example, what are the most effective bioassay techniques? How can the results of bioassays be interpreted in terms of eutrophication of surface waters in general and of Lake Tahoe in particular? How can the level of consequences to be expected for a given level of rate limiting nutrient or substance be estimated? From rational analysis and from results presented in the First Progress Report it is evident that the illumination of such questions must proceed as a part of the plan of study.

Limited success has been achieved in providing a quantitative definition of the term eutrophication. As a qualitative concept it signifies the increasing biological production in such bodies of water as lakes, reservoirs, sluggish streams, and estuaries as enrichment of such waters advances. But if solutions to the problems associated with productivity are ever to be developed, a quantitative description of degrees of eutrophication must be provided, as well as a capability developed to predict rates of eutrophication.

It is well known that nutrients such as nitrogen and phosphorus are required for algal growth. However, the question remains, is nitrogen or phosphorus, alone or in concert, usually or frequently regulating to the growth of aquatic organisms in most receiving waters. To reduce effectively the rate of eutrophication of our natural waters this question must be answered. It is not enough to remove nitrogen and phosphorus from waste waters and hope that this alone will reduce the growth rate of standing crop of plankton and attached plants in the waters of concern.

Before proceeding with a discussion of specific studies, attention is hereinafter directed to theoretical and conceptual considerations.

THE PROBLEM OF ARRAY

To develop a realistic waste management program for a body of water assumed to be excessively enriched by chemical elements such as P and N or other biostimulatory materials, it must be possible to develop facts that will permit the accomplishment of the following objectives:

1. To estimate with reasonable accuracy the background or new level of algae that will be attained from or be supported by a specific increase in concentration of nutrient.

2. To estimate the concentration of a rate limiting nutrient that will produce an algal bloom: defined as a pulse or increase in growth rate, μ of some specific value – say 2.0 day^{-1}, i.e., 200 percent increase in cell mass per day.

3. To find ways to test the possibility that more than one factor is operating simultaneously in the processes of cell growth and division, even though one factor appears to be rate limiting.

LABORATORY TEST – MODEL PROTOTYPE SIMULATION

The objective of any laboratory bioassay procedure intended to provide a basis for establishing allowable concentrations of contaminants in the environment is to provide results which apply accurately to specific practical problems. Although such an ideal is seldom achieved in practice, many laboratory tests do simulate the prototype model to an acceptable degree. Probably the best example of such an approach is the classic Biochemical Oxygen Demand (BOD) test and its use in the engineering design of waste treatment processes and in predicting the oxygen concentration (oxygen sag curve) in a river as a function of time, organic loading, and other physical and biological factors. The lack of precise simulation of the prototype is well understood; nonetheless, the laboratory assay does provide a crude approximation of the real situation which is valuable in reaching practical design solutions. A similar procedure is needed for making quantitative assessments of the biostimulation or enrichment potential of discharges of wastes having an energy (carbon) and nutrient content.

Two types of assays involving unialgal cultures have been used in laboratory tests, although neither has been developed as a standard method. These may generally be described as batch and continuous flow cultures. Both are worthy of consideration as to their efficacy in simulating the prototype and their ability to yield data which can be interpreted accurately in relation to practical situations.

FLASK CULTURE – PLUG FLOW MODEL

The traditional microbiological batch or flask culture method follows the concentration of cells with time after inoculating a substrate with some test organism. Initially the substrate has an excess of all energy sources, nutrients, and growth factors except the one substance which is assumed to be limiting to growth in the particular situation being simulated. The method is in essence a laboratory model of a continuous plug flow reactor. For an ideal plug flow system, the fluid is considered to have no longitudinal mixing and so moves through the system at a constant velocity, U, as a series of elements, or volumes, separated by impervious membranes. Such a reactor, illustrated schematically in Figure 2-1, might be simulated by a long impervious lined canal which has limited longitudinal mixing and relatively few sinks for nutrients downstream from a point of waste discharge. Theoretically, the residence time of all molecules in a plug flow system is identical: simply the reactor volume, V, divided by the throughput flow, F. The concentration, S_1, of the nutrient to be tested changes with distance (or time) in the reactor.

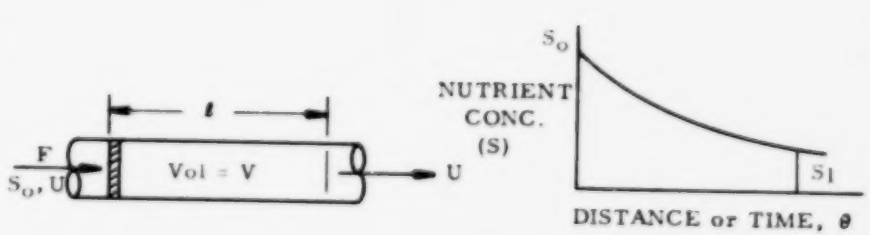


FIGURE 2-1 PLUG FLOW SYSTEM MODEL

Figure 2-2 shows a graph of the typical microbiological growth and nutrient depletion curves for a flask culture.

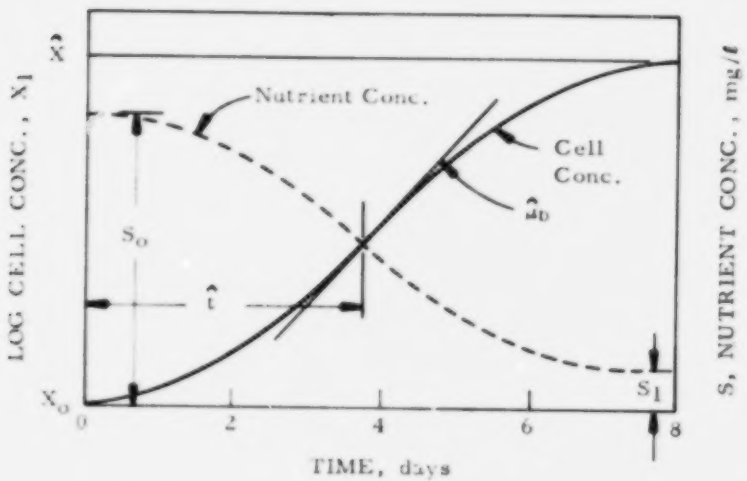


FIGURE 2-2 TYPICAL MICROBIOLOGICAL GROWTH CURVE, FLASK

There are a variety of ways to describe the characteristics of the culture's growth. One characteristic that has been suggested is the maximum cell concentration, $\hat{X}$, reached by an organism in a specific time, in a substrate with an initial concentration of S_o.

If the concern about nutrient concentration present in the environment is its effect on growth rate of specific algae; then, a better measure might be the maximum growth rate obtained with the test substrate. This characteristic is shown on Figure 2-2 as $\hat{\mu}_D$ (maximum growth rate in a flask). However, it has been well established by Michaelis-Menten [2], Monod [3], Caperon [4], Maddux [5], Williams [6], Jannasch [7], Dugdale [8], Pearson [9], and others that the specific growth rate of microorganisms is either a first order or first order-zero order (Michaelis-Menten) function of the substrate concentration. That is

$$\mu = kS \quad \text{or} \quad \mu = \frac{\hat{\mu} S}{K_s + S}$$

Where

k = first-order rate constant, time^{-1}

μ = specific growth rate $\left(\frac{\text{gm cells produced}}{\text{gm cells} - \text{day}} \right)$, time^{-1}

$\hat{\mu}$ = maximum specific growth rate, time^{-1}

K_s = nutrient concentration at one-half the maximum specific growth rate, mass per unit volume

S = rate limiting nutrient concentration, mass per unit volume.

Examination of Figure 2-2 indicates that a maximum growth rate can be estimated for the culture, but this maximum rate occurs at some undetermined, although lesser, concentration of the nutrient than existed in the original sample, S_o. This maximum growth rate should indicate the maximum rate at which the culture can utilize nutrient. However, it does not define the precise relationship between growth rate and nutrient concentration, hence an estimate of this relationship is needed.

CONTINUOUS FLOW CULTURE - (CHEMOSTATS) - CSTR MODEL

During recent years the culturing of biological populations on a continuous flow, steady state basis has been increasingly practiced. The general term Continuously Stirred Tank Reactor (CSTR) (see Figure 2-3) has been used to describe the system model; and the term chemostat applied to one such CSTR unit adapted to laboratory bioassays. The continuous flow system has the advantage that the biological population can be kept at essentially steady state conditions over a wide range of growth rates, hence the behavioral characteristics of an organism can be studied at growth rates as slow as 0.01 days^{-1}, or as rapid as 2.0 days^{-1} (1 percent to 800 percent increase in cell mass per day). This is particularly significant because gross differences in the nutritional behavior, characteristics, and even products of organism growth have been found to depend upon an organism's growth rate.

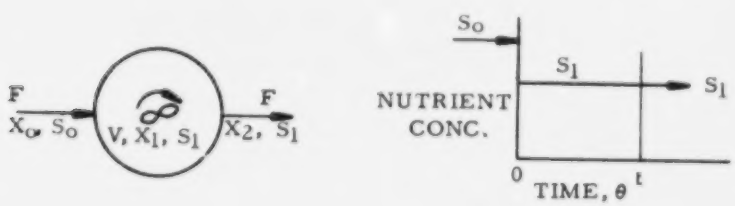


FIGURE 2-3 CSTR SYSTEM MODEL

One of the major advantages of a continuous flow-steady state assay method is that it permits the determination under laboratory conditions, of the level of standing crop of any organism or mixtures of organisms that can be supported in a given hydraulic reaction system at a specified residence time (detention period) for a known concentration of the nutrient. Moreover, the concentration of the nutrient that would produce any given increase in the growth rate (e.g., 2 times) at any specific level of cells (standing crop) can be determined from the test procedure.

KINETICS

In accordance with the previously described basis for relating growth of microorganisms to the concentration of rate limiting nutrient, it has been shown by Pearson [9] and others that the specific growth rate, μ, is best described by the Michaelis-Menten or Monod model. This is as follows:

$$\mu = \frac{\hat{\mu} S_1}{K_s + S_1} \qquad (1)$$

where the terms are as defined previously. This relationship is first-order with respect to the rate limiting nutrient concentration, S_1, at low levels of nutrient concentration; that is:

$$\mu = kS_1 \quad , \quad \text{when } S_1 \to 0 \quad . \tag{2}$$

If a substrate and cellular materials balance on a simple chemostat reactor system (CSTR) is made the following expressions for substrate and cellular growth, respectively, are obtained.

$$q = \frac{F(S_o - S_1)}{X_1 V} = \frac{S_o - S_1}{X_1 \theta} \tag{3}$$

where

q = specific nutrient removal velocity

$$\frac{\text{gms nutrient removed}}{\text{gms cells} - \text{day}} \ (\text{day}^{-1})$$

F = flow rate

S_o = influent concentration of rate limiting nutrient

S_1 = concentration of rate limiting nutrient in the reaction system

X_1 = concentration of cells in reaction system

V = volume of reaction system

θ = hydraulic residence time of system, i.e., $\theta = V/F$

and,

$$\frac{FX_2}{X_1 V} = \frac{1}{\theta_c} = \mu - k_d \tag{4}$$

where the new terms are as follows:

X_2 = concentration of cells leaving the reaction system

θ_c = cellular residence time (i.e., mean cell age in system)

$\frac{1}{\theta_c}$ = net cellular growth rate

k_d = specific decay rate $\left(\text{i.e., } \frac{\text{gms cells destroyed}}{\text{gm cells} - \text{day}} \right)$.

It can be shown that Equations 1 and 4 can be written

$$q = \frac{\hat{\mu} S_1}{Y(K_S + S_1)} \tag{5}$$

$$\frac{FX_2}{X_1 V} = \frac{1}{\theta_c} = Yq - k_d \tag{6}$$

where

$$Y = \text{yield coefficient} \left(\text{i.e., } \frac{\text{gms cells produced}}{\text{gm nutrient removed}} \right) .$$

This can be deduced from a simple continuity or dimensional analysis as follows:

$$\mu = Y \times q \tag{7}$$

$$\frac{\text{gms cells produced}}{\text{gms cells} - \text{day}} = \frac{\text{gm cells produced}}{\text{gm nutrient removed}} \times \frac{\text{gms nutrient removed}}{\text{gms cells} - \text{day}} .$$

Thus, by conducting a series of three steady state experiments for each nutrient and organism, the magnitude of the rate and system constants, $\hat{\mu}$, K_s, Y, and k_d can be determined from analysis of data collected for each of the measurable parameters, F, S_o, S_1, X_1, X_2, and O. The graphical data processing technique has been described in detail by Pearson [9]. Each of the three experiments must be at a different residence time, which will result in a different effluent concentration S_1 for a particular level of nutrient supply.

It should be readily apparent that if the constants $\hat{\mu}$, and K_S in Equation 1 are determined the specific growth rate for any steady state level of nutrient concentration in the system can be predicted. That is

$$\mu = \frac{\hat{\mu} S_1}{K_s + S_1} . \tag{1}$$

This information coupled with the determined value of the constants Y and k_d permits estimation of the net growth rate of the system, $1/\theta_c$.

Using Equation 3 and substituting μ/Y for q

$$\mu = \frac{Y(S_o - S_1)}{X_1 \theta} \tag{8}$$

transposing

$$X_1 = \frac{Y(S_o - S_1)}{\theta \mu} . \tag{9}$$

The relationship between the mean cell concentration X_1 and the residence time θ for a given nutrient level concentration is then obvious.

The kinetic description of a continuous flow system and its application to eutrophication problems can be summarized as follows:

1. A given level of rate limiting nutrient (i.e., $S_1 = NO_3^-$, $PO_4^{\equiv}$, etc.) in the receiving water determines the specific growth rate, μ, that can be supported by that rate limiting concentration [i.e., $\mu = f(S_1)$].

2. For a given level of S_1 and μ and yield constant, Y, the mean cell concentration, X_1, (standing crop) is determined by the residence time in the system (Equation 9).

5. The net or gross cellular growth rate that will follow from any level of nutrient concentration can be estimated once the rate constants and coefficients have been determined for the organism and nutrient form (Equation 4).

It appears that this is the type of information needed for prediction of allowable levels of organisms (algae) and nutrients of concern in the receiving water. Moreover, these data are susceptible to laboratory evaluation. The accuracy of such predictions (simulation of the prototype), however, can only be assessed by determination in the laboratory of the kinetic characteristics described herein, followed by assessment of the resulting conditions in the field.

CHAPTER III

MATERIALS AND METHODS

INTRODUCTION

As noted in Chapter I (Scope of Report), two basic types of assay methods – batch and continuous culture – were used in the laboratory assay of Lake Tahoe water with and without added nutrients. The batch method was conducted in flasks; and the continuous flow technique was used both in laboratory chemostats and in outdoor experimental ponds in which the results of laboratory assays were tested under conditions simulating Lake Tahoe situations. Samples of waste water effluents and other sources of nutrients used in the laboratory and pond assays were collected, treated, and analyzed in the manner hereinafter described. The type of test alga used is likewise described in this chapter. For reasons of clarity the special apparatus and specific procedures used in flask, chemostat, and pond assays are described in subsequent chapters devoted specifically to such assays.

SOURCE OF SAMPLES

The various sources of samples used for assays included surface runoff from three land areas in differing stages of development, sewage effluents, and Lake Tahoe water. Ward, Incline, and Upper Truckee-Trout creeks were chosen as examples of surface runoff from relatively undeveloped, newly developed, and well-established urbanized land areas. Examples of waste water discharges chosen for assay included raw sewage and primary, secondary, and tertiary effluents from the treatment plant of the South Tahoe Public Utilities District. Primary and activated sludge (secondary) effluents were also obtained from the Reno-Sparks sewage treatment plant, which serves areas that draw their water supply from the Truckee River, hence indirectly from Lake Tahoe. Samples of Lake Tahoe water were obtained from two sampling stations, one located in a deep portion of the lake, the other near the north shore.

COLLECTION OF SAMPLES

All samples, with the exception of Lake Tahoe water from the north shore sampling station, which was obtained by a shoreline pump and pipeline system, were collected either in 1-gal glass jugs or 5-gal polyethylene containers. Most of the samples assayed were "grab" samples collected by a technician; however, composite samples of raw sewage and primary, secondary, and tertiary effluents were collected specifically for use in the flask assay studies. These composite samples were obtained with an automatic sampler which collected an aliquot of the source each hour for 24 hours. Throughout the sampling period, the containers were housed in a refrigeration unit. Containers were generally washed in the laboratory and rinsed with the sample at the time of collection. All samples were collected at regular intervals; the containers were segregated, labeled, and used only for collection of samples from one specific source.

PREPARATION OF SAMPLES

Preliminary preparation of samples for physical and chemical analyses varied somewhat depending on the specific method chosen for each assay. Samples selected for flask culture, including Lake Tahoe water used for dilution, were filtered through Whatman glass fiber filter pads (GF/C) and finally through HA Millipore(R) filters (0.45 μ pore size). They were then stored in tightly covered polyethylene containers and frozen immediately, unless the test was to begin within five days. Samples for chemostat assay were treated in a similar manner. Due to the large

quantity of water required for pond studies, secondary effluents used in the continuous flow pond assays could not be filtered by the Millipore technique. Instead they were filtered through a cotton wad to remove large bits of debris and sediments which might clog the input tubing. Tertiary effluent, also assayed in pond studies, was found to be relatively free of suspended matter or debris and hence was not filtered before use. An attempt was made to filter the Lake Tahoe water used in pond studies by means of a diatomaceous earth, swimming pool filter. The attempt was unsuccessful, however, because the head loss through the filter increased the total dynamic head of the system to a value beyond the range of the pump.

Each sample brought to the laboratory for assay was analyzed chemically for a number of constituents. Aliquots of samples reserved for this purpose were filtered through the glass fiber and Millipore filters previously described, except for that portion used in BOD and COD examinations. Samples were kept in tightly capped 2-ℓ polyethylene containers and stored in a refrigerator at temperatures approaching 0°C until all chemical determinations were completed.

ANALYTICAL PROCEDURES

Chemical analyses were made according to Standard Methods [10] in determining BOD, COD, pH, alkalinity, NH_3, chlorides, and conductivity. Methods described by Strickland and Parsons [11] were considered more suitable for iron, NO_2, NO_3, and reactive inorganic phosphorus at the low concentrations prevailing in the Tahoe samples. Similarly, procedures recommended by Jenkins [12], were found more appropriate for soluble organic phosphorus and soluble organic nitrogen. Details of individual analyses and precision of analyses are presented in Appendix A.

The technique for measuring total suspended solids (SS) and volatile suspended solids (VSS) was patterned from a combination of the procedures outlined in Standard Methods [10] 1965; Strickland and Parsons [11] 1965; and Maciolek [13] 1962. Whatman glass fiber filters (GF/C) were used in solids separation. The filters were prepared by soaking in distilled water to wash the fibers free of salts. They were then placed in a muffle furnace for 30 minutes at 450°C to destroy any organic matter present without fusing the glass fibers. After cooling, the filters were dried in a hot air oven at 75°C and tared quickly on a Mettler semi-micro balance, to avoid error due to the extremely hydroscopic nature of the dried filter. In making the solids determinations the sample was applied to the filter until it was completely clogged and the volume of filtrate noted. The filter pads with their load of suspended solids were then dried overnight at 75°C and the dry weight recorded to the nearest 0.01 mg. They were then stored in marked envelopes in a refrigerator freezer. Suspended solids values were later redetermined by redrying and reweighing. Thereafter the filters were ignited at 550°C for 2 hours, soaked with a few drops of distilled water to rehydrate the mineral matter, dried overnight at 75°C, and weighed. The difference between the SS weight and the weight after ignition was then used to determine the VSS in mg/ℓ.

In some cases it was necessary to revise the suggested methods in order to expand the scope of the analysis to encompass the wide range of nutrient concentrations encountered in the various samples assayed. The approach was to prepare two standard curves for the Beckman spectrophotometer, one using a 1-cm pathway cuvette and the other a 5-cm cuvette. For some determinations, the range of concentrations using the two pathway cells was from 1 μg/ℓ to 2000 μg/ℓ. Samples were diluted with deionized water prior to analysis when the level of nutrients exceeded the maximum which could be determined by the calibration curves.

TEST ORGANISMS

The alga Selenastrum gracile (Reinsch) was selected and utilized as a test organism for assays on the basis of consideration of the characteristics of an ideal test organism and of favorable results reported by Skulberg [14] with the same genus. It has the disadvantage of producing large cells under rich nutrient conditions.

Also, newly formed cells tend to remain attached, but because they rarely exceed four in a group, cells are easily distinguished for counting. For the conditions pertaining at Tahoe these limitations were considered to be minor.

A basic culture of S. gracile was maintained for seeding of assays. It was grown on a solution of 10 percent Skulberg's medium, which is specifically designed for the culturing of Selenastrum, after experience proved it to be superior to modified Bristol's medium (used in the study prior to August 1968). The algal cells were harvested for use when they had reached the log phase of growth, i.e., when the cells were in good physiological condition. Maintenance of the log phase of growth of the culture at first posed some problems. Continual transfer of test cells to a freshly prepared nutrient medium was the initial procedure. However, new crops of cells were often slow in reaching log growth rate and it was difficult to correlate the desired commencement date for assay with the proper growth phase of the test cells. Furthermore, algal contamination of the stock culture during the transfer process was a common occurrence. A continuous culture method was therefore applied (θ = 5 days) to maintain the population of test cells at a constant growth rate and to avoid contamination.

CHAPTER IV

FLASK ASSAYS

PROCEDURE AND APPARATUS

All samples to be assayed by the flask culture method, including Lake Tahoe water used for dilution, were sterilized by aspectic filtration through HA Millipore(R) filters (0.45 μ pore size). Glassware employed in conjunction with the assay was dry heat sterilized to avoid bacterial contamination.

In making the assay of any nutrient source, the filtered sample was first diluted to the desired concentration with filtered Lake Tahoe water. 150 *ml* of this dilution was then placed in each of five sterile 250 *ml* Erlenmeyer flasks. Cells of S. gracile in good physiological condition were centrifuged and washed twice in Lake Tahoe water to minimize nutrient carry over from the stock culture to the test flasks. The concentration of S. gracile in the final suspension was calculated either by measurement of the light extinction of the chloroplasts (670 mμ) or, in later experiments, by counting under the microscope. An equal volume of the suspended cells was then added to each test flask, so that the concentration of cells in the 150 *ml* of liquid was approximately 50 cells/mm^3.

The flasks were then closed with foam plugs. It was found, however, that although these plugs had good air diffusion properties the foam decayed with time and shed debris into the cultures. Therefore, the method was abandoned in favor of loose fitting plastic beakers inverted over the tops of the flasks.

The inoculated test flasks were placed in a 20°C constant temperature room and incubated on a gently moving (30 cycles/min) shaker table (see Figure 4-1) for a period of five days. Illumination of approximately 170 foot-candles (1830 lux) intensity was provided by a pair of 30 watt G. E. fluorescent lamps, No. 30T8-CW, Coolwhite, three feet in length.

The cell concentration in the test flasks were determined by cell counts at the end of 1, 3, and 5 days during a five-day period of incubation. After the final counts were completed, a suspended solids measurement was made on a composite of the liquid in the five replicate flasks.

At the time specified for counting cells, a 10 *ml* aliquot was taken from each flask and centrifuged for 10-15 minutes at 2000 rpm. After centrifugation, 8-9 *ml* of the supernatant were removed with a Pasteur pipette and the pellet of cells resuspended in the remaining liquid medium. A drop of the suspension was then put on a Spencer Bright-Line hemocytometer for counting under the microscope. Duplicate counts were made for each flask and five replicates were performed for each concentration; thus a total of 30 counts was made for each concentration of sample tested.

The duplicate counts for each flask were averaged, and the resulting values were then averaged to obtain a mean count for the five replicates constituting the assay. By plotting these data as in Figure 2-2 (Chapter II) and observing the maximum slope of the curve, values of the mean maximum growth rate, $\hat{\mu}_0$, (hereinafter designated as "maximum growth rate") were obtained for each of the test flasks, as well as for the mean of the five replicates. A second value of the maximum growth rate, designated as $\hat{\mu}_{0\ell}$, was obtained for each assay, by determining the log growth equation of best fit to the 1, 3, and 5-day cell count data by the method of least squares. In each assay the maximum number of cells attained at the end of the growth period, $\hat{\chi}_5$, was observed. Thus the cell concentration data were used to express the growth response of S. gracile in three different ways, $\hat{\mu}_0$, $\hat{\mu}_{0\ell}$, and $\hat{\chi}_5$.

Cell mass, and the time required to reach maximum growth rate, were likewise observed. For comparative purposes, volatile suspended solids (VSS), or total concentration of biological mass, and chlorophyll produced were determined at the end of five days of growth.

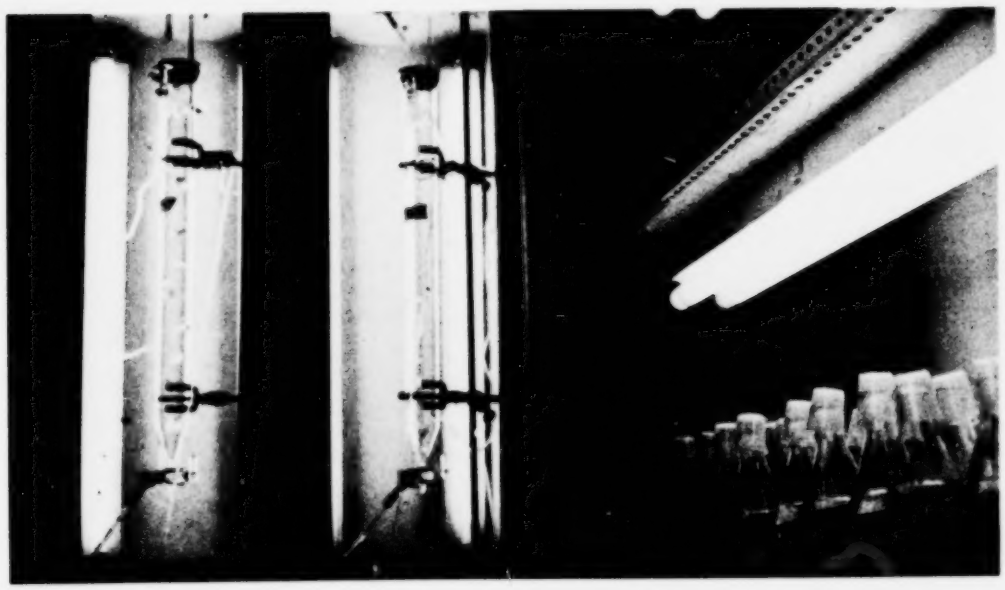


FIGURE 4-1 CULTURE FLASKS ON ILLUMINATED SHAKER TABLE (ALSO CHEMOSTATS)

Some estimate of the normal nitrogen and phosphorus content of algal cells was deemed necessary for an interpretation of the results. Table 4-1 presents values for various species of algae (not including S. gracile). Because of "luxury consumption," a behavior characteristic of algae, i.e., an ability to accumulate nitrogen and phosphorus, the table was used largely as a guide to judge the reasonableness of data. From data presented by Gerloff and Skoog [15] it was estimated that luxury consumption begins to appear when the nitrogen concentration is in the 9-10 mg N/ℓ range. The culture medium utilized in growing the stock culture of S. gracile for bioassays contained 10.2 mg N/ℓ, whereas some of the samples assayed were nitrogen poor. Therefore, washing of cells with distilled water prior to transfer to assay cultures was used to minimize the effect of carry over of nutrients to test samples.

Utilizing the apparatus and procedures described above, 767 assays were made of samples from various nutrient sources undiluted and diluted with Lake Tahoe water to yield concentrations adopted in the experimental design. The general type of sample and the number of bioassays completed of each type are shown in Table 4-2.

TABLE 4-1

NITROGEN AND PHOSPHORUS CONTENT OF VARIOUS ALGAE [15]

Material analyzed	Percent of dry weight	
	N	P
Microcystis sp.*	9.27	0.52
Anaebaena sp.*	8.27	0.53
Microcystis aeruginosa+	6.83	0.69
Aphanizomenon sp.#	10.05	--
Aphanizomenon sp.§	--	0.6
Anaebaena cylindrica//	6.95-9.0	--
Oscillatoria sp.//	5.65-7.42	--
Oscillatoria brevis	7.45	1.28
Anabaena sp.	4.04	1.20
Lyngbya hemensii	7.46	1.07
Spirogyra sp.//	3.81	--
Chlorella sp.¶	1.4-11.0	--
Chlorella vulgaris**	8.0	1.1
Chlorella vulgaris//	1.89-4.34	--
Chlorella pyrenoidosa++	--	0.94-1.5
Scenedesmus bijugatis	4.2-6.1	0.53-1.8
Stigeoclonium stagnatile##	6.52	1.89
Calothrix sp.	4.39	1.18
Tribonema sequale//	1.74-2.95	--
Monodus subterraneus//	1.22-2.94	--
Stream periphyton§§	3.29	--

*Birge and Juday (1922).
+Gerloff and Skoog (1954).
#Prescott (1960).
§Phinney and Peek (1961).
//Fogg and Collyer (1953).
¶Spoehr and Milner (1949).
**Geoghegan (1953).
++Scott (1943).
##Bogan et al. (1960).
§§Kevern and Ball (1965).

TABLE 4-2

SOURCE OF SAMPLES AND NUMBER OF FLASK ASSAYS

Source of Sample	Number of Assays
Lake Tahoe	75
Surface Runoff	195
Sewage Effluents	357
Experimental Ponds	80
Indian Creek Reservoir	30
Modified Skulberg Medium	30
TOTAL	767

Growth rates, $\hat{\mu}_1$, $\hat{\mu}_{1,t}$, and cell concentrations, $\hat{X}_m$, for the first five of the six sources shown in the table were in turn compared statistically, analyzed for seasonal variation, and interpreted in terms of nutrient concentration versus growth response. The results are presented in the following sections of this chapter, as are the results of Solids and Cell Mass Determinations. Assays of Skulberg medium were made to check the viability of the standard culture, and are not herein discussed.

RESULTS

A. GROWTH RATES, $\hat{\mu}_1$, $\hat{\mu}_{1,t}$, AND CELL CONCENTRATIONS, $\hat{X}_m$

1. Lake Tahoe Water (LTW)

Because water from Lake Tahoe was used as the diluent in all assays requiring reduction in concentration of the sample, LTW from both the near shore and the mid-lake sampling stations were assayed monthly. The results were analyzed for four principal purposes:

a. To determine the ability of the lake water to support algal growth

b. To identify possible seasonal variations in the growth stimulating character of LTW

c. To reveal any nutritional differences between the near shore and deep portions of the lake

d. To detect differences in Lake Tahoe and the creeks flowing into it.

Observed maximum growth rates and maximum concentration of *S. gracile* cells attained at the end of five days of flask culture are shown in Table 4-2 for both shore and mid-lake samples. From the table it is evident that the coefficient of variation, or the relative variance of replicate assays, fluctuated appreciably for both the maximum growth rate, $\hat{\mu}_1$, and the cell concentration, $\hat{X}_m$. Because $\hat{\mu}_1$ is calculated from data often including the values of $\hat{X}_m$, this indicates a wide variation in the accuracy of the cell counting technique.

The apparent time for the biomass to double itself may be obtained from the values of $\hat{\mu}_1$ shown in Table 4-2 by dividing them into the natural logarithm of 2 (i.e., 0.693). From such a calculation a range of from 1.1 to 2.3 days results for the shore samples; and from 0.9 to 2.2 days at mid-lake.

Table 4-2 shows that the maximum growth rate, $\hat{\mu}_{1,t}$, computed by regression analysis of cell counts versus time, fluctuated in much the same manner as did $\hat{\mu}_1$ and $\hat{X}_m$, although the values tended to be smaller than the corresponding values of $\hat{\mu}_1$. The correlation coefficient, however, was appreciably higher than the 0.672 value necessary to indicate a 99.9 percent level of probability, hence it may be concluded that the growth rate value indicated by $\hat{\mu}_{1,t}$ were highly significant.

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TABLE 4-5

MAXIMUM GROWTH RATES AND MAXIMUM CELL CONCENTRATIONS ATTAINED AT THE END OF FIVE DAYS IN FLASK CULTURES OF LTW

Sampling Date	Maximum Growth Rate ($\hat{\mu}_1$, day^{-1})				Maximum One-Day Rate ($\hat{\mu}_{1,1}$, day^{-1})				Maximum Cell Concentration ($\hat{\lambda}_5$, cells/mm^3)			
	Mid-Lake Sample	Coef. Var. (%)	Shore Sample	Coef. Var. (%)	Mid-Lake Sample	Corr. Coef.	Shore Sample	Corr. Coef.	Mid-Lake Sample	Coef. Var. (%)	Shore Sample	Coef. Var. (%)
6/15/68	0.408	36.7	0.494	21.0	0.678	0.880	0.755	0.881	274.8	12.6	255.4	19.8
7/13/68	0.410	49.0	0.582	15.6	0.700	0.736	0.935	0.955	194.2	42.6	151.6	8.4
8/ 9/68	0.550	16.0	-	-	0.293	0.864	-	-	168.2	19.7	-	-
9/15/68	0.512	17.0	0.504	45.5	0.718	0.955	0.779	0.909	230.4	47.7	199.6	29.5
10/ 4/68	0.559	41.2	0.614	57.5	0.849	0.881	0.847	0.750	62.4	25.8	126.0	14.4
11/ 8/68	0.550	50.6	0.560	8.6	0.190	0.788	-	-	119.8	50.2	144.2	22.5
12/ 3/68	0.396	14.8	0.468	49.5	0.548	0.887	0.790	0.811	108.6	51.7	57.8	22.5
1/ 3/69	0.470	57.8	0.570	40.7	0.144	0.811	0.651	0.875	69.0	10.0	104.0	25.0

Statistical Comparisons. Tables 4-4 and 4-5 present a statistical assessment of maximum growth rates $\hat{\mu}_1$ and $\hat{\mu}_{1,1}$ for shore and mid-lake samples ranked according to ascending magnitude rather than chronological sequence. Table 4-6 presents a similar comparison and assessment of values of maximum cell concentration, $\hat{\lambda}_5$. In all three tables the underlines bracket values that are not significantly different at a 95 percent confidence level.

From a comparison of the three tables it is evident that the three cell growth measures ($\hat{\mu}_1$, $\hat{\mu}_{1,1}$, and $\hat{\lambda}_5$) differ considerably in ranked position. For example, the largest value of $\hat{\mu}_1$ (0.796) observed was in the mid-lake sample of 12-3-68, whereas the shore sample of that date yielded the smallest (57.8) value of $\hat{\lambda}_5$. Or considering only the mid-lake sample of that date, it ranked 15th in the $\hat{\mu}_1$ series and 10th in the $\hat{\lambda}_5$ series. This variation in ranking between cell growth measures, evident throughout Tables 4-4, 4-5, and 4-6, probably means that the measurements reflect different types of algal response to the nutrients in LTW. As to which of the three is the most interpretable measure of flask assay results, the statistical evidence favors $\hat{\lambda}_5$ and $\hat{\mu}_{1,1}$ because at a confidence level of 95 percent the 15 samples represent 8 ranges of values of these measures, as compared to only 6 ranges for the 15 values of $\hat{\mu}_1$. This is to say that in the ideal situation each of the 15 values would be in itself statistically significant, whereas complete failure of the assay method would be signaled by no significant difference between the smallest and the largest value observed. Thus 15 ranges (underlines) would represent the most sensitive assay possible; and one range, the least sensitive.

The specific meaning of the underlines, or brackets, in Table 4-4, for example, is that all values of growth rate in the range of 0.270 to 0.454 days^{-1} are statistically from a single sample population. Observed values in the 0.510 to 0.512 range, however, are not distinguishable statistically from any value in the range down to 0.324; and so on. To aid the reader in a clear understanding of this concept, and in interpreting the results of assays presented in Tables 4-4, 4-5, and 4-6, as well as similar tables which follow in this chapter, Table 4-7 is presented. Here both the growth rates, $\hat{\mu}_1$, and their $\log_e 2$ reciprocals are presented.







TABLE 4-7

SUMMARY OF FLASK ASSAYS OF *S. gracile* IN LAKE TAHOE WATER

Maximum Growth Rate, $\hat{\mu}_t$ ($days^{-1}$)	Time to Double Cell Mass		Cell Concentration	
	Apparent $0.693/\hat{\mu}_t$ (days)	Calculated at 95% Confidence (days)	Apparent, $\hat{X}_5$ ($cells/mm^3$)	Calculated at 95% Confidence ($cells/mm^3$)
0.270	2.6		57.6	
0.302	2.3		82.4	57.6 - 69.0
0.324	2.1 +		69.0	
0.338	2.1		104.0	82.4 - 126.0
0.350	2.0	2.6 - 1.5	119.8	69.0 - 144.2
0.368	1.9		126.0	
0.372	1.86	2.1 - 1.51	144.2	104.0 - 168.6
0.428	1.6		151.6	
0.434	1.6	2.0 - 1.26	168.2	119.8 - 195.6
0.510	1.36	1.9 - 1.25	168.6	
0.512	1.35		194.2	168.2 - 230.4
0.550	1.26	1.5 - 1.1	195.6	
0.592	1.25		230.4	194.2 - 255.8
0.634	1.1		255.8	230.4 - 276.0
0.720	0.9	1.1 - 0.9	276.0	

In terms of calculated time to double cell mass, for example, the table means that any observed value in the range of 1.5 to 2.6 days is no more significant than any other value in this range. If the observed value is in the range of 1.35 to 1.5 days, it is no more significant than any other up to the limit of 2.1 days; if it is between 1.26 and 1.35 days, it might quite as well be 2.0 days; and so on. A similar interpretation of cell concentration values is appropriate to Tables 4-5 and 4-6.

Seasonal Variations. The seasonal variation in the maximum growth rate and maximum cell concentration of *S. gracile* in LTW is shown in Figure 4-2. The same general trend is shown by the values of $\hat{\mu}_t$, $\hat{\mu}_{t\ell}$, and $\hat{X}_5$. However, $\hat{\mu}_t$ and $\hat{X}_5$ show a wider range of fluctuation, which indicates that they may be more sensitive to variations in the nutrient content of the lake water. There are inadequate data to show a definite seasonal cycling, but it appears that $\hat{\mu}_t$, $\hat{\mu}_{t\ell}$, and $\hat{X}_5$ values for mid-lake samples were approaching a low point that would occur sometime in January 1969. A similar low occurred in January 1968.

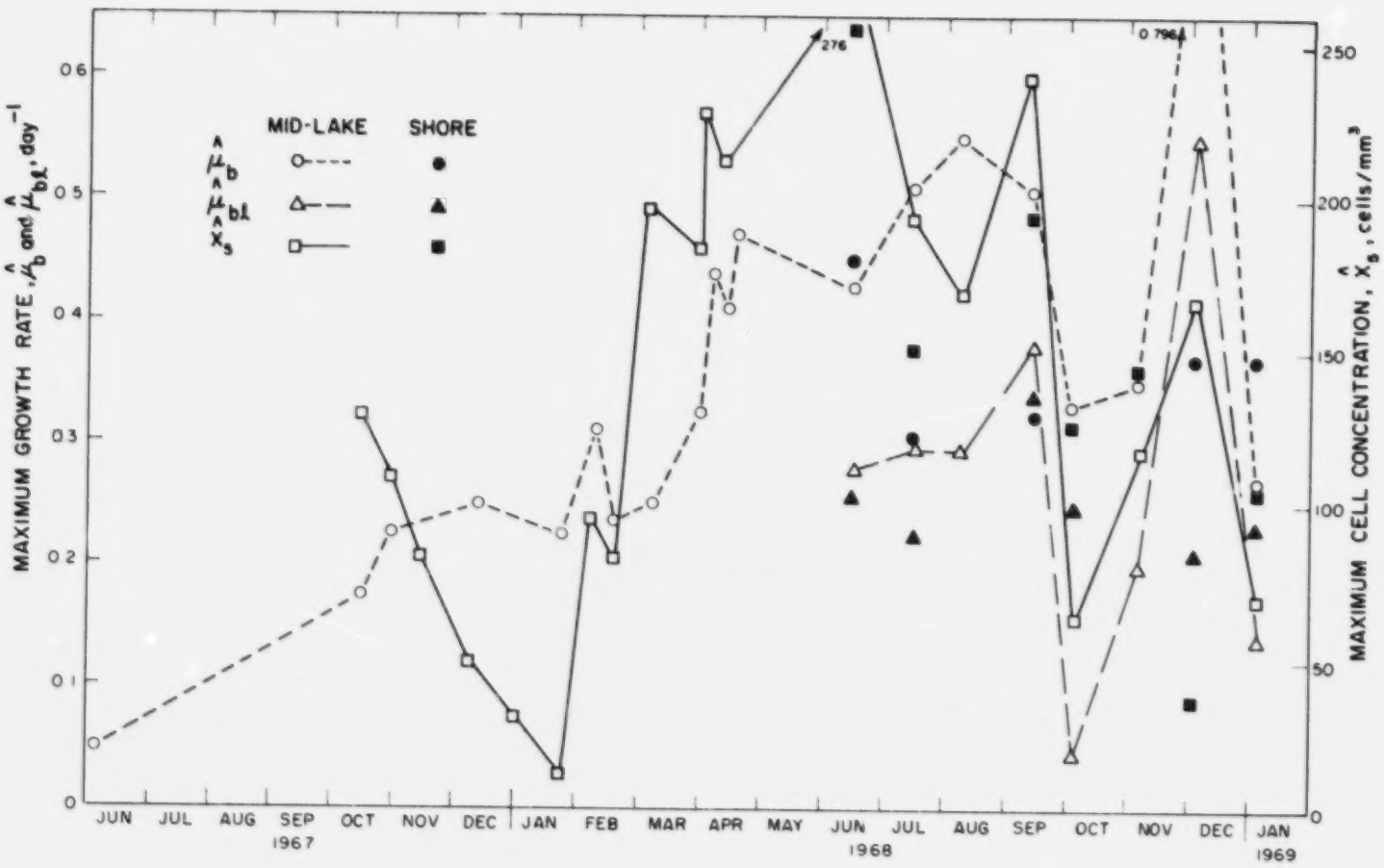


FIGURE 4-2 SEASONAL VARIATION IN THE MAXIMUM GROWTH RATE AND MAXIMUM CELL CONCENTRATION ATTAINED AT END OF FIVE DAYS IN FLASK CULTURE IN LTW

Nutrient Concentration vs Growth Parameters. The results of chemical analyses of LTW are presented in Appendix B. They show a wide fluctuation in the composition of LTW which can only be attributed to the difficulties of making precise measurements of low concentrations of chemicals by the methods of analysis currently in use. At the same time the experimental error in making cell counts at the concentrations supported by small amounts of nutrients is equally well known. Consequently, when nutrient concentration was compared with growth parameters such as $\hat{\mu}_b$, $\hat{\mu}_{b\ell}$, and $\hat{X}_5$ there appeared to be little correlation.

2. Surface Runoff

Systematic sampling of surface runoff during the period of study herein reported was confined to three creeks, each representing land areas in different stages of development. Ward, Incline, and Upper Truckee-Trout creeks were selected as representing, respectively, surface runoff from relatively undeveloped, newly developed, and well-established urbanized land areas.

Tables 4-8, 4-9, and 4-10 summarize the algal growth response parameters; $\hat{\mu}_b$, $\hat{\mu}_{b\ell}$, and $\hat{X}_5$; attained in various sample concentrations of creek waters assayed. The coefficient of variation for the five replicate assays of each concentration are also shown in Tables 4-8 and 4-10 for $\hat{\mu}_b$ and $\hat{X}_5$. Table 4-9 shows the correlation coefficient for the regression analyses used to compute $\hat{\mu}_{b\ell}$.

It is noteworthy that the variation within the five replicates was less for the maximum cell concentration, $\hat{X}_5$, than in the maximum growth rate, $\hat{\mu}_b$, for practically all creek assays. Again, this supports the earlier suggestion that $\hat{X}_5$ is possibly a more sensitive indicator of response to changes in nutrient concentrations than is $\hat{\mu}_b$.

Unfortunately, it is impossible directly to compare the precision of the values of $\hat{\mu}_{b\ell}$ with $\hat{\mu}_b$ and $\hat{X}_5$. However, it seems logical that a value computed from 15 data points would yield a better estimate of the maximum growth rate for the population than would the mean of five individual calculations.

The correlation coefficients for the regression analyses indicated a 99.9 percent level of significance ($r \geq 0.749$) for all sample concentrations with the exception of those concentrations yielding growth rates of less than 0.10 day^{-1}. In general, the values of $\hat{\mu}_{b\ell}$ less than 0.20 day^{-1} yielded lower correlation coefficients. However, less precision at lower growth rates is to be expected, because small differences at low cell concentrations introduce much greater variability.

The excellent fit of the data to the log growth curve shown by the high values of correlation coefficient indicates that the equation describes the growth in the flasks when adequate nutrients and carbon sources are present. Rapid growth in assay flasks containing high concentration of nutrients resulted in carbon dioxide limiting conditions; thereby, impeding growth near the end of the fifth day. This caused a considerable proportion of the variability in $\hat{X}_5$, and also affected the regression analysis when computing $\hat{\mu}_{b\ell}$. The values of $\hat{\mu}_b$ generally were not affected by carbon dioxide limiting conditions, because the section of the growth curve showing a maximum rate of increase was selected to calculate $\hat{\mu}_b$. The maximum rate of cell increase usually occurred within the third day of the experiment. A further discussion of this point appears in Chapter VII. However, it is possible that greater rates of growth would have occurred between the third and fifth days if carbon limiting conditions had not developed.

TABLE 1-7

MAXIMUM DAILY RATE, $\hat{R}$, ATTAINED DURING FIVE DAYS IN PLAIN CULTURE OF UNDER WATER

Sampling Date	Number of Samples	Number of Samples					
		Water Column		Ice-Water Column		Sediment-Water Column	
		Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)	Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)	Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)
1/23/1978	1					0.0197	0.0
	2					0.0175	10.1
	3					0.0191	8.4
1/24/1978	1	0.0191	0.0	0.0191	0.0		
	2	0.0178	7.1	0.0191	0.0		
	3	0.0178	7.1	0.0191	0.0		
1/25/1978	1					0.0197	0.0
	2					0.0175	10.1
	3					0.0191	8.4
2/ 1/78	1	0.0191	0.0				
	2	0.0178	7.1				
	3	0.0178	7.1				
2/ 2/78	1			0.0191	0.0		
	2			0.0191	0.0		
	3			0.0191	0.0		
2/27/78	1	0.0178	0.0	0.0191	0.0	0.0178	0.0
	2	0.0178	0.0	0.0191	0.0	0.0178	0.0
3/13/78	1	0.0191	0.0	0.0191	0.0	0.0191	0.0
3/27/78	1	0.0191	0.0	0.0191	0.0	0.0191	0.0
	2	0.0191	0.0	0.0191	0.0	0.0191	0.0
4/ 7/78	1	0.0178	0.0	0.0191	0.0	0.0178	0.0
	2	0.0178	0.0	0.0191	0.0	0.0178	0.0

TABLE 1-8

MAXIMUM DAILY RATE, $\hat{R}_B$, AND CARBON ASSIMILATION COEFFICIENT, α, IN EACH CULTURE OF UNDER WATER

Sampling Date	Number of Samples	Number of Samples					
		Water		Ice-Water		Sediment-Water	
		Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)	Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)	Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)
1/23/1978	1					0.0197	0.0
	2					0.0175	10.1
	3					0.0191	8.4
1/24/1978	1	0.0191	0.0	0.0191	0.0		
	2	0.0178	7.1	0.0191	0.0		
	3	0.0178	7.1	0.0191	0.0		
1/25/1978	1					0.0197	0.0
	2					0.0175	10.1
	3					0.0191	8.4
2/ 1/78	1	0.0191	0.0				
	2	0.0178	7.1				
	3	0.0178	7.1				
2/ 2/78	1			0.0191	0.0		
	2			0.0191	0.0		
	3			0.0191	0.0		
2/27/78	1	0.0178	0.0	0.0191	0.0	0.0178	0.0
	2	0.0178	0.0	0.0191	0.0	0.0178	0.0
3/13/78	1	0.0191	0.0	0.0191	0.0	0.0191	0.0
3/27/78	1	0.0191	0.0	0.0191	0.0	0.0191	0.0
	2	0.0191	0.0	0.0191	0.0	0.0191	0.0
4/ 7/78	1	0.0178	0.0	0.0191	0.0	0.0178	0.0
	2	0.0178	0.0	0.0191	0.0	0.0178	0.0

TABLE 1-9

Sampling Date	Number of Samples	Number of Samples					
		Water Column		Ice-Water Column		Sediment-Water Column	
		Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)	Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)	Maximum Rate ($\mu g C \cdot m^{-3} \cdot day^{-1}$)	Coef. Var. (%)
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						
	1						
	2						
	3						

Statistical Comparisons. Comparisons were made for only the 50 and 100% sample concentrations of creek waters because earlier assays [1] at lower concentrations, 10% and lower, had shown that changes in growth characteristics were undetectable at these levels. Even the 50% sample concentration assays often showed that the differences were undetectable. Much of this lack of sensitivity can be attributed, however, to the seasonal variation of the diluent, LTW, noted in a previous section.

Tables 4-11 and 4-12 show the maximum growth rates, $\hat{\mu}_0$ and $\hat{\mu}_{0\ell}$, corresponding sample source, and statistical significance of results from assays of 50 and 100% sample concentrations for creek samples ranked in ascending order of magnitude of $\hat{\mu}_1$. As previously discussed in relation to Table 4-4, all values connected with a common underline are not significantly different at the 95% probability level. It is evident from Table 4-11 that all samples diluted to 50% concentration with LTW either showed a reduction in $\hat{\mu}_0$ and $\hat{\mu}_{0\ell}$ or did not differ significantly from the 100% sample concentration assay.

Table 4-13 shows a similar comparison of the cell concentration of S. gracile attained at the end of 5 days in flask culture for the same creek samples and dilutions reported in Tables 4-11 and 4-12. It shows that the reduction in growth due to diluting the sample with LTW to a 50% concentration is more evident in $\hat{X}_5$ values than in values of $\hat{\mu}_1$. Cell concentrations either decreased or did not differ when the sample was diluted, with the exception of the 10-4-68 sample from Truckee-Trout Creek which showed a greater value of $\hat{X}_5$ at the 50% than at the 100% concentration. This increase might possibly be attributed to the reduction of some unidentified toxic effect present in the sample; however, the rate of growth as shown in Tables 4-9 and 4-11 apparently was not affected. Why such a contrast should develop is unknown unless an experimental error occurred.

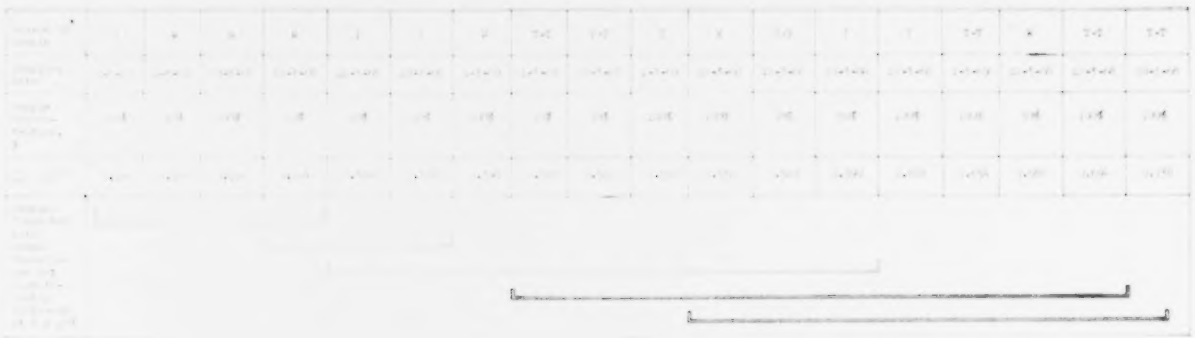
Tables 4-14, 4-15, and 4-16 show the maximum growth rates, $\hat{\mu}_0$ and $\hat{\mu}_{0\ell}$, and cell concentrations, $\hat{X}_5$, and statistical significance of the results of assaying 100% sample concentrations of the creek waters. They show that growth response varied widely with the time of sampling as well as from creek to creek. When compared with Tables 4-13 and 4-11 it is clear that a smaller difference is necessary for statistical significance at 100% concentration than at 50%. This is mainly due to the higher reproducibility obtained in the 100% assays which yields a smaller variance for computing the significant differences.

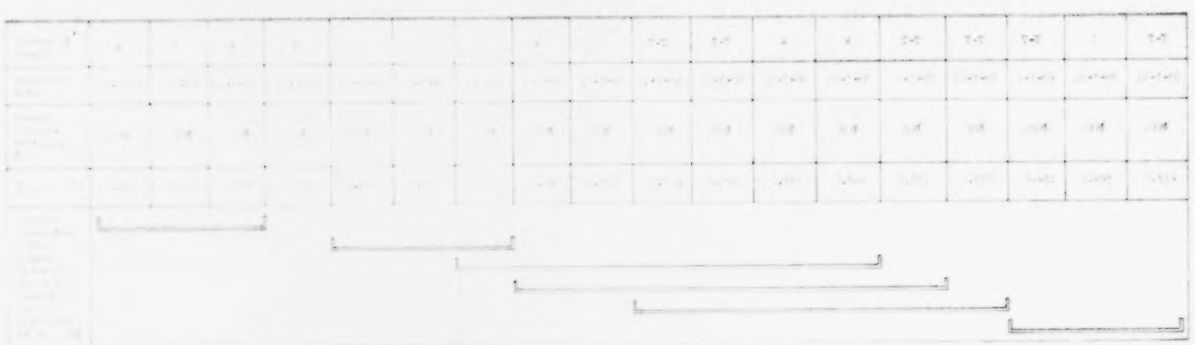
When either $\hat{\mu}_0$, $\hat{\mu}_{0\ell}$, or $\hat{X}_5$ is used to show the variability of response, it appears that Truckee-Trout and Incline creeks differ very little as measured by the flask assay. Because both areas are relatively heavily populated, it is not surprising that the growth response of the two creeks is essentially the same.

The response in Ward Creek was generally lower than that found for the other two creeks, with the exception of the sample collected on 4 November 1968. The relatively large values of $\hat{\mu}_0$ (3.96) observed at that time could have been caused by the early rain or snowfall which melted and cleared the drainage area of the nutrients accumulated over the spring and summer. This explanation is supported by high values for all creeks at that date, with decline in the growth response to low levels in December 1968 and in January 1969.

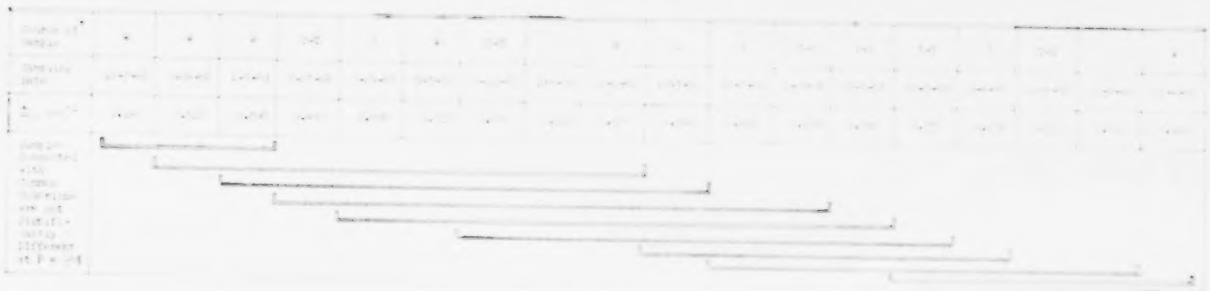
Seasonal Variation. The seasonal variation in the maximum growth rates and cell concentration of S. gracile in 100% sample concentrations of creek waters is shown in Figure 4-6. The variation in growth response with time as shown by the three growth parameters ($\hat{\mu}_0$, $\hat{\mu}_{0\ell}$, and $\hat{X}_5$) is essentially the same for Ward Creek. However, for Truckee-Trout and Incline creeks only the $\hat{\mu}_{0\ell}$ and $\hat{X}_5$ values show a similar variation. The pattern for the values of $\hat{\mu}_0$ differs significantly. This indicates that in this case $\hat{\mu}_0$ is perhaps not the most sensitive value for detecting differences in growth response.

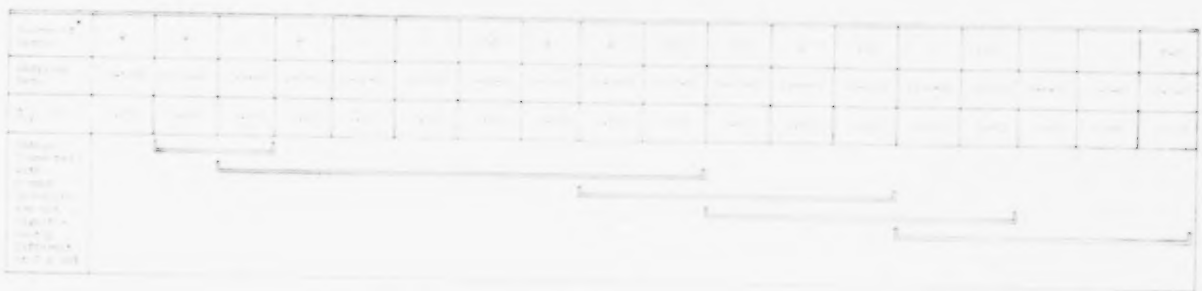
Statistical comparisons of the growth response in the creeks measured in terms of $\hat{X}_5$ and based upon the analysis presented in Table 4-16 shows that Ward Creek exhibited a lower growth response than Truckee-Trout Creek in all months sampled













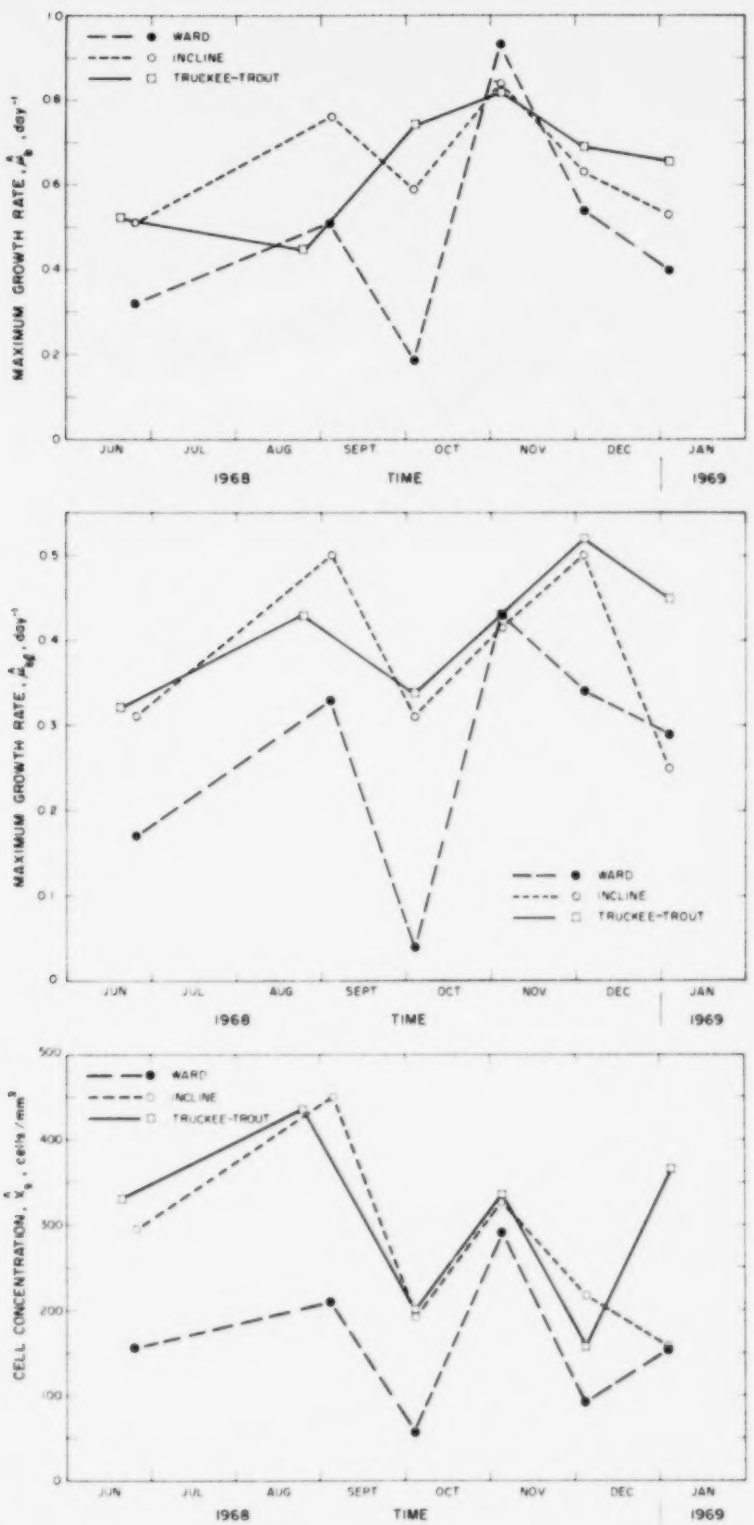


FIGURE 4-3 SEASONAL VARIATION IN THE MAXIMUM GROWTH RATES, $\hat{\mu}_0$ AND $\hat{\mu}_{opt}$, AND CELL CONCENTRATION, $\hat{X}_0$, FOR 100% SAMPLE CONCENTRATIONS OF VARIOUS CREEK WATERS

except November 1968. Ward Creek did not differ significantly (95 percent significance level) from Incline Creek in November 1968 and January 1969. Truckee-Trout and Incline creeks differed significantly during December and January. There was a large increase in growth response in Truckee-Trout Creek between December and January while the response for the other two creeks was declining or remaining relatively constant. It is unlikely that this surge was caused by temporal changes, because there is little difference in the weather in December and January. Probably a change in quality of some discharge to the creek occurred during this period.

A comparison of the growth response in the various creeks measured by $\hat{\mu}_{t\ell}$ show statistical differences in response similar to those measured by $\hat{X}_5$, but do not show the same trend. For example, the December samples for Incline and Truckee-Trout creeks show a decrease in growth when measured by $\hat{X}_5$, but an increase is shown when the measure is the growth rate $\hat{\mu}_{t\ell}$. The maximum growth rate, $\hat{\mu}_t$, also shows essentially the same statistical variation when comparing the individual creeks; however, the variations are less pronounced than those shown by $\hat{X}_5$ and $\hat{\mu}_{t\ell}$ (Figure 4-3).

Figure 4-4 shows the variation with time of $\hat{\mu}_t$ for 50 percent concentrations of creek samples. Because of the large influence exerted by the diluting water (LTW), the trends and response shown by these assays often differed from those of the 100 percent concentration. Therefore, temporal changes in the creeks were less discernible in the 50 percent sample concentrations and tend to show only their effect upon LTW, or vice versa. This tendency is also shown in the statistical comparison of the 50 percent and 100 percent concentrations of sample in Tables 4-11 and 4-12.

Nutrient Concentration vs Growth Response. Chemical analyses, including nutrient concentrations, for the various surface runoff (creek) samples assayed in flask culture are presented in Appendix B. Figure 4-5 shows the observed variation in growth response, as measured by $\hat{\mu}_t$, $\hat{\mu}_{t\ell}$, and $\hat{X}_5$, in relation to the total nitrogen concentration initially in the flask culture. In the range of nitrogen concentration between approximately 100 and 500 $\mu g/\ell$ (Figure 4-5) the variation appears to be linear. Below 100 $\mu g/\ell$, however, there is little identifiable relationship between growth rate and total nitrogen. A similar relationship is evident for $\hat{\mu}_{t\ell}$ and total nitrogen concentration if the change in scale of the abscissa in the figure is taken into consideration. Again, the critical concentration appears to be approximately 100 $\mu g/\ell$ of total nitrogen. This indicates that once the total nitrogen exceeds 100 $\mu g/\ell$, the growth rate rapidly approaches its maximum value; and if growth is to be limited in samples having more than 100 $\mu g/\ell$ total nitrogen, some nutrient other than nitrogen must be in short supply.

The $\hat{X}_5$ values appear roughly to be linearly related to the total nitrogen concentration. The apparent wide variation in relationship between cell concentration attained in 5 days and the concentration of total nitrogen can be explained partly by the change in scale of the abscissa in Figure 4-5. If plotted on the same abscissa scale used for $\hat{\mu}_t$, the data for $\hat{X}_5$ appears in a linear relationship as good or better than that shown for $\hat{\mu}_t$ and $\hat{\mu}_{t\ell}$.

In Figure 4-6 is shown the variation between maximum growth rates and cell concentration in relation to the concentration of total phosphorus initially in the culture flask during the assay of various creek waters. From the figure it appears that once the total phosphorus concentration exceeds approximately 20 $\mu g/\ell$ there is adequate phosphorus for the maximum growth rate to be attained. In many of the assays a 10 $\mu g/\ell$ concentration was all that was required for cell growth to approach the maximum rate attainable.

Based upon the foregoing values of total nitrogen and phosphorus required to attain or approach the maximum growth rate, it is possible to estimate values of K_s (Chapter II), a widely used measure of growth response which represents the concentration of limiting nutrient required for the growth rate to equal one-half the maximum possible value of $\hat{\mu}_t$. From the data presented in Figures 4-5 and 4-6 it appears that a reasonable estimate of K_s (based upon S_o, or initial, concentrations) would be about 225 $\mu g/\ell$ of nitrogen and 20 $\mu g/\ell$ of phosphorus for $\hat{\mu}_t$ and 150 $\mu g/\ell$ of nitrogen and 20 $\mu g/\ell$ of phosphorus for $\hat{\mu}_{t\ell}$.

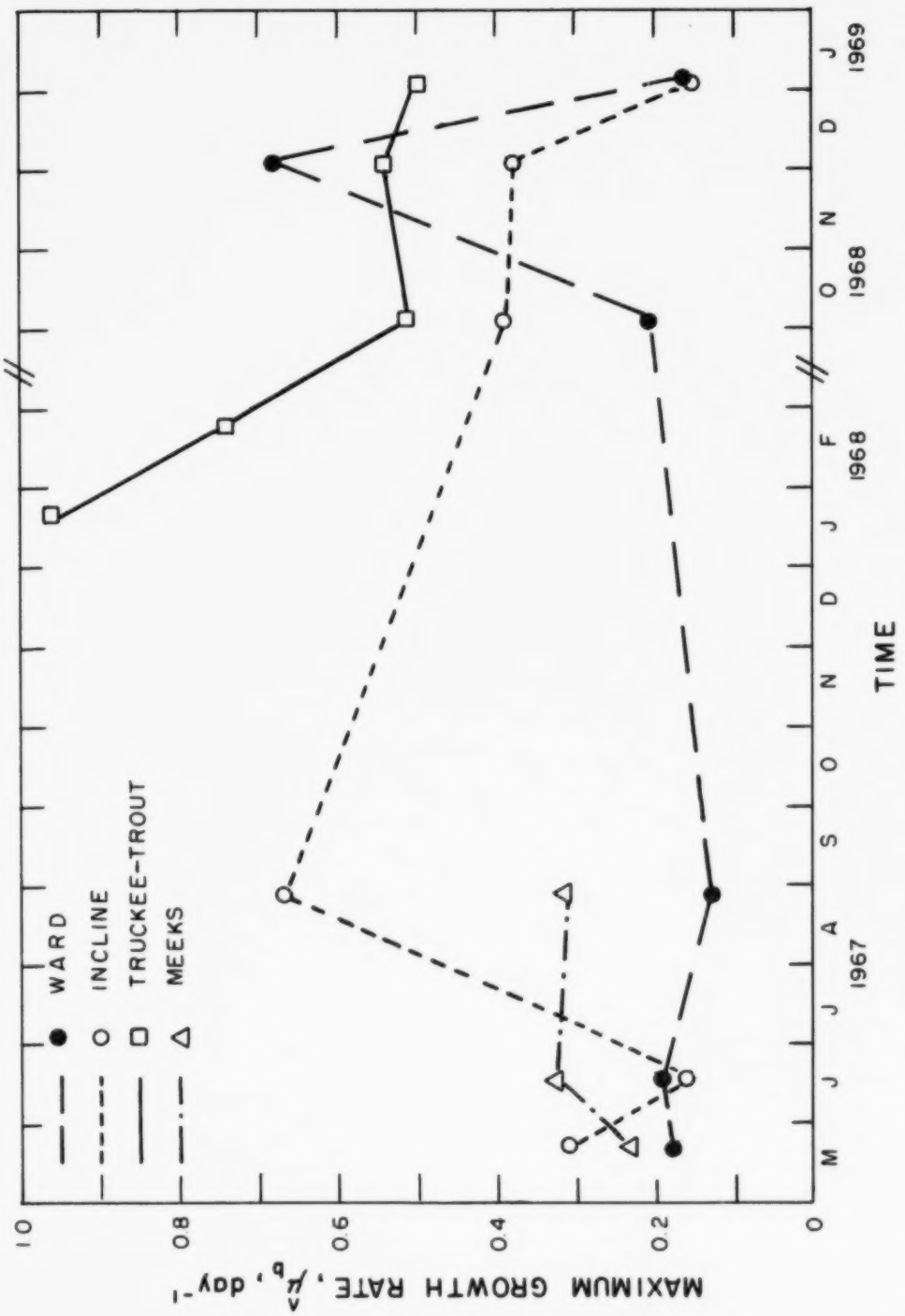
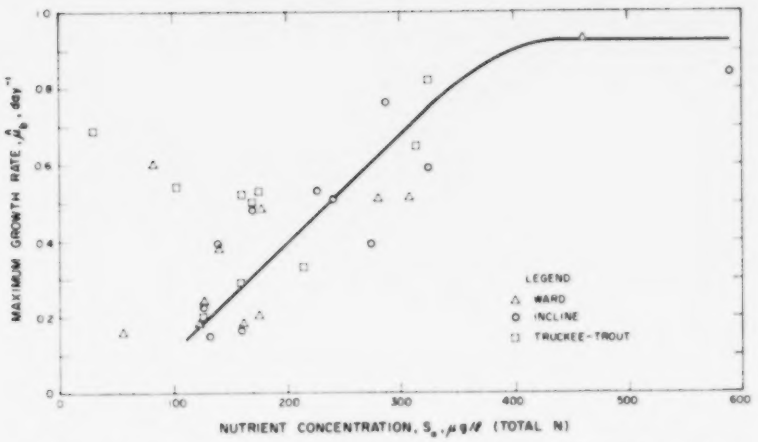
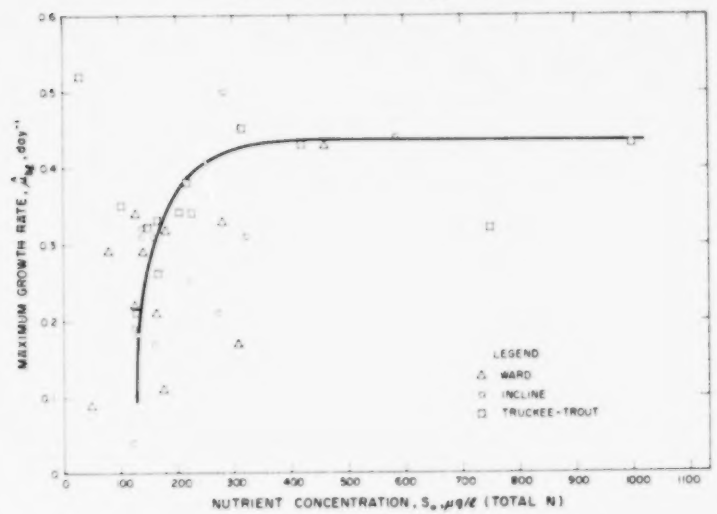


FIGURE 4-4 SEASONAL VARIATION IN THE MAXIMUM GROWTH RATE, $\hat{\mu}_b$, FOR THE 50% CONCENTRATION OF VARIOUS CREEK WATERS





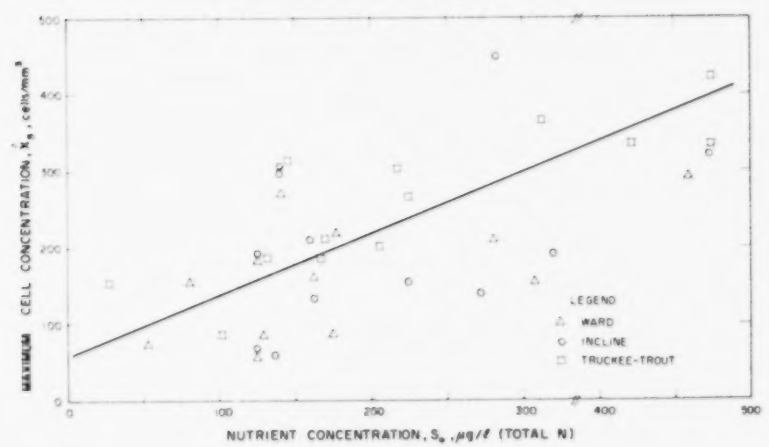


FIGURE 4-4 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_0$ AND $\hat{\mu}_M$, AND CELL CONCENTRATIONS, $\hat{X}_o$, WITH CONCENTRATION OF TOTAL NITROGEN IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT

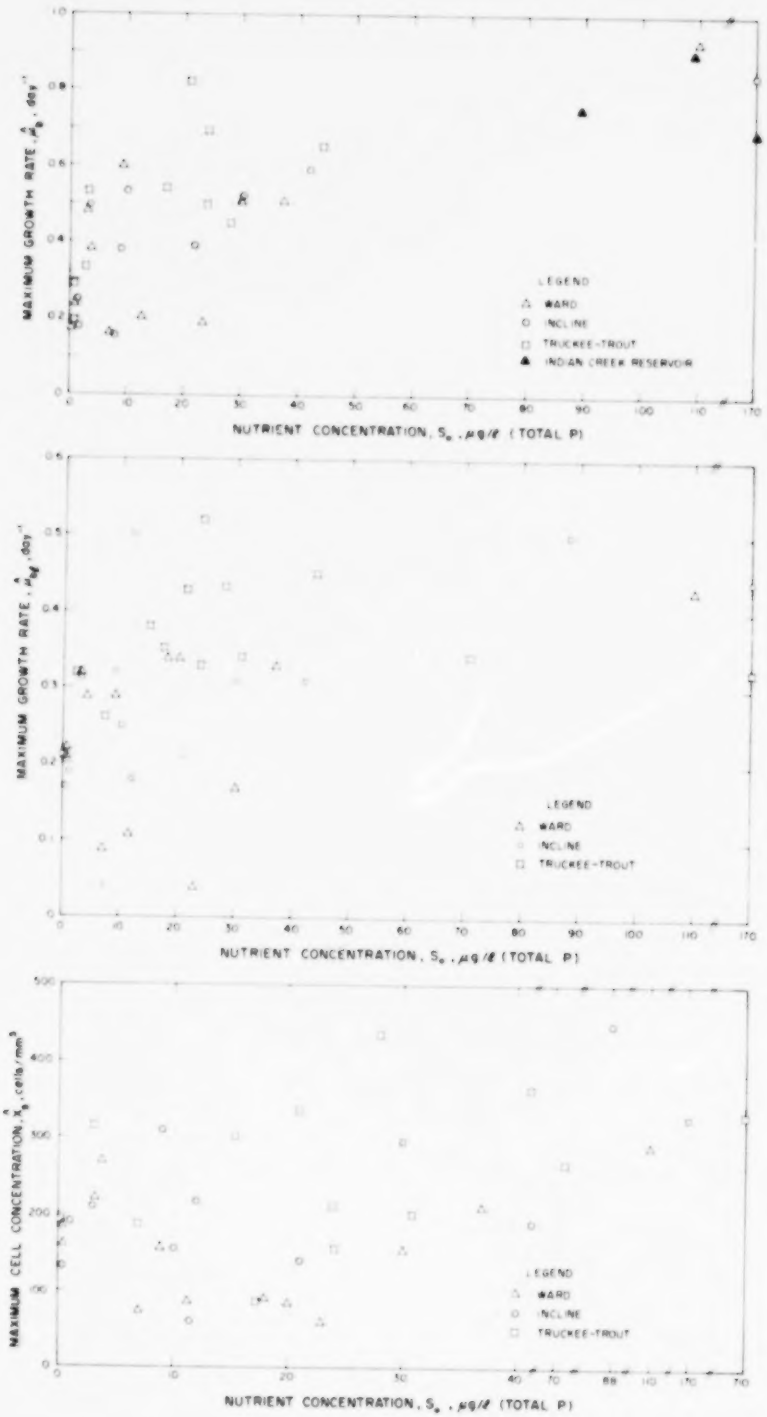


FIGURE 4-6 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{bf}$, AND CELL CONCENTRATIONS, $\hat{X}_b$, WITH CONCENTRATION OF TOTAL PHOSPHORUS IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT

Plots for various forms of nitrogen and phosphorus, and reactive iron, similar to those presented for total nitrogen and phosphorus are presented in Appendix C (Figures C-1 through C-7). Similar interpretations can be made for these figures. However, total nitrogen and total phosphorus were selected as examples because these measures give the highest degree of correlation between nutrient and cell growth response.

In the case of iron, Goldman [16] has reported that iron is one of the growth limiting substances in Lake Tahoe. Based upon the data summarized in Figure C-7, it appears that a K_s value for reactive iron would be approximately 5 $\mu g/\ell$. This means that if the reactive iron concentration is greater than 5 $\mu g/\ell$, the growth rate would be expected to approach the maximum, provided some other substance does not become the growth limiting factor.

3. Waste Water Effluents

For the batch assays herein reported, samples were collected only from the South Tahoe Public Utilities District (STPUD) Waste Water Reclamation Plant. The decision to limit sampling to this particular installation was based on the unique opportunity the plant afforded through year-round operation of a multistage treatment process. Thus it was intended to obtain comparative data in relation to seasonal variations and degree of treatment of waste water.

The results of 60 separate flask assays of STPUD samples are reported in Tables 4-17, 4-18, and 4-19. The maximum growth rates, $\hat{\mu}_b$ and $\hat{\mu}_{b\ell}$, and cell concentration, $\hat{X}_s$, attained at the end of five days; together with the coefficient of variation for $\hat{\mu}_b$ and $\hat{X}_s$ and the correlation coefficient for $\hat{\mu}_{b\ell}$, are reported for each of the sample concentrations assayed.

As noted for the LTW and creek assay data, the assessment of growth response using the cell concentration, $\hat{X}_s$, data appears to be more precise than that measured by $\hat{\mu}_b$. The coefficients of variation for assay results in which five replicate assays for each concentration were performed (Sample #4S, 5S, and 6S) were smaller or equivalent for the $\hat{X}_s$ data in 42 of 56 assays.

Highly significant (99 percent confidence level) correlation coefficients were obtained for the least squares fit of the cell concentration data to the log growth equation, the exception being the 10 percent tertiary effluent sample collected on 6 August 1968 which produced a value of 0.5759. This deviation is attributable to a failure to dechlorinate the sample before introducing it into the assay flask. The other concentrations were not affected because the large dilution factor at 0.1 percent and 1.0 percent concentrations overcame the effect of chlorine in the sample. Data from assays with 0.1 percent sample concentrations in general showed the lowest correlation coefficients. This was also observed for the assays of 0.1 percent creek water.

Statistical Comparisons

A split-plot design was used to analyze the results of waste water assays. As previously noted (Procedure and Apparatus) all values ($\hat{\mu}_b$, $\hat{\mu}_{b\ell}$, and $\hat{X}_s$) used to express the growth response are directly related, but the reproducibility of each method of calculation is not the same. Tables 4-20 through 4-37 were prepared to show the variation within individual samples and between various concentrations of sample for the three methods of expressing growth response.

Table 4-20 presents a comparison and a statistical assessment of ranked values of maximum growth rate, $\hat{\mu}_b$, observed at three concentrations (0.1%, 1.0%, and 10%) for the waste water sample designated Sample #4S in Table 4-17 (6 August 1968 sampling date). As in previous tables the underlines bracket the growth rate values, $\hat{\mu}_b$, and the percentage concentrations which are not significantly different at a 95 percent confidence limit. The only sample showing a significantly different





growth rate was the 10 percent concentration of tertiary effluent, which exhibited the exceedingly low rate of 0.196 day^{-1} because of the presence of chlorine in the sample. The 0.1 percent and 10 percent concentrations of primary effluent differed significantly from each other, but neither differed from the 1 percent concentration assay.

Table 4-21 presents a similar summary and statistical significance comparison of the three sample concentrations tested (0.1%, 1.0%, and 10%) for the four effluent samples. It shows that only the 10 percent concentration of tertiary effluent differed significantly from the other 10 percent sample concentration assays. This is explained by the presence of chlorine.

Table 4-22 presents a summary and statistical comparison of ranked values of the maximum cell concentration, $\hat{X}_5$, observed at the three concentrations of Sample #43. This comparison of growth responses is the same as shown in Table 4-20, with the exception that $\hat{X}_5$ rather than $\hat{\mu}_0$ is used to show the growth response. The 10 percent tertiary effluent assay again shows the effect of not dechlorinating the sample. The $\hat{X}_5$ values, however, tend to show differences in growth response that were not detectable by the $\hat{\mu}_0$ values.

Table 4-23 evaluates the $\hat{X}_5$ data of Table 4-22 in relation to concentration of the sample assayed. When compared with Table 4-21, the results indicate that $\hat{X}_5$ is a somewhat more discriminatory measure than $\hat{\mu}_0$. Discounting the low (78.8) value of $\hat{X}_5$ for tertiary effluent on the basis of chlorine toxicity, Table 4-23 shows that at the 1 percent concentration tertiary effluent had a significantly greater ability to support algal growth than did either raw, primary, or secondary effluents; and that the latter three do not differ significantly one from the other. Further, at the 10 percent concentration secondary and primary effluents are equally capable of stimulating algal growth which is significantly greater than that of raw sewage. This indicates that the nutrients in raw sewage may be unavailable to algae.

Table 4-24 presents a summary and statistical significance comparison of ranked maximum growth rates, $\hat{\mu}_{0\ell}$, observed at three sample concentrations (0.1%, 1.0%, and 10%) for Sample #43. The table was prepared by comparing the slopes of the least squares regression analyses at the 95 percent confidence level to determine significant differences. When compared with Table 4-20 ($\hat{\mu}_0$) and Table 4-22 ($\hat{X}_5$) it is evident that the growth of S. gracile as measured by $\hat{\mu}_0$ and $\hat{\mu}_{0\ell}$ is quite similar, with $\hat{X}_5$ appearing to be the most sensitive of the three methods in assessing differences in growth response.

Table 4-25 presents a summary of the $\hat{\mu}_{0\ell}$ values, similar to Table 4-24. Inspection of the table reveals that only the 10 percent concentration assays for raw sewage and tertiary effluent show a significant difference in biostimulatory response. However, the tertiary data must be discounted as before; and again the comparative inability of raw sewage at the 10 percent concentration to support growth is revealed.

Tables 4-26, 4-28, and 4-30 present a comparison – including a statistical assessment – of maximum growth rates, $\hat{\mu}_0$ and $\hat{\mu}_{0\ell}$, and cell concentrations, $\hat{X}_5$, attained at the end of five days in flask culture, observed at three sample concentrations (0.1%, 1.0%, and 10%) for each effluent sample collected on 23 October 1968 (Sample #52). Tables 4-27, 4-29, and 4-31 present a similar summary and statistical significance comparison of the three sample concentrations tested (0.1%, 1.0%, and 10%) for the same waste water samples. Inspection of Tables 4-26, 4-28, and 4-30 reveals that all three methods of expressing growth response show a significant difference in all three sample concentrations for the four effluent samples which was not observed in Sample #43 (Tables 4-20, 4-22, and 4-24). It is likely that these differences were detectable because of improvements in laboratory technique gained from experience in performing assays.

TABLE V-40

MAXIMUM GROWTH RATE, $\hat{\mu}$, CORRESPONDING CAMPLA CONCENTRATION AND STATISTICAL SIGNIFICANCE FOR TOTAL WATER WATER EFFLUENTS (SAMPLE #40)

Source of Sample	$\hat{\mu}$, day^{-1}; Sample Concentration, (%), and Statistical Significance: 5% Significant Difference at 95% Confidence Level
Raw Sewage	2.455 (10%) 2.454 (5.1%) 1.801 (1%)
Primary Effluent	2.204 (5.1%) 2.108 (1%) 1.894 (10%)
Secondary Effluent (A.S.)	2.385 (5.1%) 2.254 (1%) 2.023 (10%)
Tertiary Effluent	2.254 (10%) 2.385 (5.1%) 2.023 (1%)

TABLE V-41

MAXIMUM GROWTH RATE, $\hat{\mu}$, FOR THE RAW SEWAGE SAMPLE, AT VARIOUS ALGAE CONCENTRATIONS AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (%) FOR TOTAL WATER WATER EFFLUENTS (SAMPLE #40)

Sample Concentration	$\hat{\mu}$, day^{-1}; Source of Sample and Statistical Significance: No Significant Difference at 95% Confidence Level
5.1%	2.385 (III) 2.385 (II) 2.204 (I) 2.254 (B)
1%	2.108 (II) 2.023 (III) 1.801 (B) 1.894 (I)
10%	2.254 (III) 2.455 (B) 2.204 (II) 2.108 (I)

Code

(B) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE V-42

MAXIMUM CELL CONCENTRATION, $\hat{N}$, ATTAINED AT END OF FIVE DAYS IN FLASK CULTURE, CORRESPONDING SAMPLE CONCENTRATIONS AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (%) FOR TOTAL WATER WATER EFFLUENTS (SAMPLE #40)

Source of Sample	$\hat{N}$, cells/m^3; Sample Concentration, (%), and Statistical Significance: No Significant Difference at 95% Confidence Level
Raw Sewage	550.0 (5.1%) 550.0 (1%) 550.0 (10%)
Primary Effluent	550.0 (5.1%) 550.0 (1%) 550.0 (10%)
Secondary Effluent (A.S.)	550.0 (5.1%) 550.0 (1%) 550.0 (10%)
Tertiary Effluent	550.0 (10%) 550.0 (5.1%) 550.0 (1%)

TABLE V-43

MAXIMUM CELL CONCENTRATION, $\hat{N}$, ATTAINED AT END OF FIVE DAYS IN FLASK CULTURE, CORRESPONDING SAMPLE ORIGIN, AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (%) FOR TOTAL WATER WATER EFFLUENTS (SAMPLE #40)

Sample Concentration	$\hat{N}$, cells/m^3; Source of Sample and Statistical Significance: No Significant Difference at 95% Confidence Level
5.1%	550.0 (B) 550.0 (I) 550.0 (III) 550.0 (II)
1%	550.0 (II) 550.0 (B) 550.0 (I) 550.0 (III)
10%	550.0 (III) 550.0 (B) 550.0 (II) 550.0 (I)

Code

(B) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE 4-2a

MAXIMUM GROWTH RATE, $\hat{\mu}_{pf}$, CORRESPONDING SAMPLE CONCENTRATION AND STATISTICAL SIGNIFICANCE FOR CITRUS WASTE WATER EFFLUENTS (SAMPLE #43)

Source of Sample	$\hat{\mu}_{pf}$, day^{-1}; Sample Concentration, (%), and Statistical Significance: No Significant Difference at 95% Confidence Level
Raw Sewage	0.580 (0.1%) 0.452 (10%) 0.567 (1.0%)
Primary Effluent	0.419 (0.1%) 0.536 (1.0%) 0.404 (10%)
Secondary Effluent (A.S.)	0.418 (0.1%) 0.519 (1.0%) 0.582 (10%)
Tertiary Effluent	0.268 (10%) 0.492 (0.1%) 0.404 (1.0%)

TABLE 4-2

MAXIMUM GROWTH RATE, $\hat{\mu}_{pf}$, CORRESPONDING SAMPLE SOURCE, AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (%) FOR CITRUS WASTE WATER EFFLUENTS (SAMPLE #43)

Sample Concentration	$\hat{\mu}_{pf}$, day^{-1}; Source of Sample and Statistical Significance: No Significant Difference at 95% Confidence Level
0.1%	0.580 (R) 0.536 (III) 0.519 (II) 0.419 (I)
1%	0.567 (R) 0.519 (II) 0.536 (I) 0.404 (III)
10%	0.268 (III) 0.452 (R) 0.582 (II) 0.404 (I)

(R) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE V-26

MAXIMUM GROWTH RATES, $\hat{\mu}_i$, ATTAINED AT END OF FIVE DAYS IN FLASK CULTURES, CORRESPONDING CARRYING CAPACITIES, AND STATISTICAL SIGNIFICANCES OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (I) FOR RAW WASTE WATER EFFLUENT (SAMPLE #3)

Source of Sample	$\hat{\mu}_i$, day^{-1}; Sample Concentration, (%), and Statistical Significances in Significant Difference at 95% Confidence Level		
Raw Sewage	.454 (.1%) (II)	.440 (1%) (I)	.415 (10%) (III)
Primary Effluent	.431 (.1%) (II)	.420 (1%) (I)	.405 (10%) (III)
Secondary Effluent (A.S.)	.415 (.1%) (II)	.400 (1%) (I)	.390 (10%) (III)
Tertiary Effluent	.400 (.1%) (II)	.390 (1%) (I)	.380 (10%) (III)

TABLE V-27

MAXIMUM GROWTH RATES, $\hat{\mu}_i$, CORRESPONDING CARRYING CAPACITIES, AND STATISTICAL SIGNIFICANCES OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (I) FOR RAW WASTE WATER EFFLUENT (SAMPLE #3)

Sample Concentration	$\hat{\mu}_i$, day^{-1}; Source of Sample and Statistical Significances in Significant Diff--ence at 95% Confidence Level			
.1%	.454 (II)	.440 (III)	.415 (I)	.454 (R)
1%	.440 (III)	.440 (I)	.440 (II)	.440 (R)
10%	.440 (I)	.440 (R)	.440 (II)	.440 (III)

Code

(R) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE V-28

MAXIMUM CELL CONCENTRATIONS, $\hat{C}_i$, ATTAINED AT END OF FIVE DAYS IN FLASK CULTURES, CORRESPONDING GROWTH RATES, AND STATISTICAL SIGNIFICANCES OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (I) FOR RAW WASTE WATER EFFLUENT (SAMPLE #3)

Source of Sample	$\hat{C}_i$, cells/cm^3; Sample Concentration, (%), and Statistical Significances in Significant Difference at 95% Confidence Level		
Raw Sewage	1,000.0 (.1%) (II)	970.0 (1%) (I)	917.0 (10%) (III)
Primary Effluent	970.0 (.1%) (II)	930.0 (1%) (I)	897.0 (10%) (III)
Secondary Effluent (A.S.)	917.0 (.1%) (II)	897.0 (1%) (I)	1,045.0 (10%) (III)
Tertiary Effluent	897.0 (.1%) (II)	790.0 (1%) (I)	720.0 (10%) (III)

TABLE V-29

MAXIMUM CELL CONCENTRATIONS, $\hat{C}_i$, ATTAINED AT END OF FIVE DAYS IN FLASK CULTURES, CORRESPONDING CARRYING CAPACITIES, AND STATISTICAL SIGNIFICANCES OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (I) FOR RAW WASTE WATER EFFLUENT (SAMPLE #3)

Sample Concentration	$\hat{C}_i$, cells/cm^3; Source of Sample and Statistical Significances in Significant Difference at 95% Confidence Level			
.1%	970.0 (II)	897. (III)	930.0 (R)	1,000.0 (I)
1%	930.0 (III)	930.0 (II)	930.0 (R)	930.0 (I)
10%	720.0 (III)	790.0 (I)	970.0 (R)	1,000.0 (II)

Code

(R) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE V-3

MAXIMUM GROWTH RATE, Ω_2, [illegible] AND ADDITIONAL [illegible] FOR CITY WATER
WATER SYSTEMS [illegible]

Source of Sample	Ω_2, day^{-1}; range [illegible] and Statistical Significance; % Significant Difference at 95% Confidence Level [illegible]			
Raw Sewage	[illegible]	[illegible]	[illegible]	[illegible]
Primary Effluent	[illegible]	[illegible]	[illegible]	[illegible]
Secondary Effluent (A.S.)	[illegible]	[illegible]	[illegible]	[illegible]
Tertiary Effluent	[illegible]	[illegible]	[illegible]	[illegible]

TABLE V-4

MAXIMUM GROWTH RATE, Ω_2, [illegible] AND ADDITIONAL [illegible] FOR CITY WATER
WATER SYSTEMS [illegible]

Sample Concentration	Ω_2, day^{-1}; Ranges of Sample and Statistical Significance; % Significant Difference at 95% Confidence Level [illegible]			
0.1%	[illegible] (II)	[illegible] (III)	[illegible] (R)	[illegible] (I)
1%	[illegible] (III)	[illegible] (R)	[illegible] (II)	[illegible] (I)
10%	[illegible] (R)	[illegible] (III)	[illegible] (I)	[illegible] (II)

(R) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE 4-9

MAXIMUM GROWTH RATES, $\hat{\mu}_1$, OBSERVED IN SAMPLE CONCENTRATIONS AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (S) FOR DUCK WASTE WATER EFFLUENT (SAMPLE #2)

Source of Sample	$\hat{\mu}_1$, day^{-1}; Sample Concentration, (S), and Statistical Significance in Significant Differences at 95% Confidence Level: S
Raw Sewage	0.450 (0.1%) 0.430 (1%) 0.387 (10%)
Primary Effluent	0.470 (0.1%) 0.450 (1%) 0.405 (10%)
Secondary Effluent (A.S.)	0.420 (1%) 0.410 (0.1%) 0.369 (10%)
Tertiary Effluent	0.405 (0.1%) 0.375 (1%) 0.338 (10%)

TABLE 4-10

MAXIMUM GROWTH RATES, $\hat{\mu}_1$, OBSERVED IN SAMPLE CONCENTRATIONS AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (S) FOR DUCK WASTE WATER EFFLUENT (SAMPLE #2)

Sample Concentration	$\hat{\mu}_1$, day^{-1}; Source of Sample and Statistical Significance: No Significant Difference at 95% Confidence Level: S
0.1%	0.540 (R) 0.490 (I) 0.450 (III) 0.420 (II)
1%	0.470 (III) 0.430 (II) 0.405 (R) 0.390 (I)
10%	0.372 (I) 0.360 (R) 0.369 (II) 0.360 (III)

Code

(R) Raw Sewage (II) Secondary Effluent
(I) Primary Effluent (III) Tertiary Effluent

TABLE 4-5

MAXIMUM GROWTH RATE, μ_{max}, CORRESPONDING SAMPLE CONCENTRATION AND STATISTICAL SIGNIFICANCE FOR EFFLUENT WASTE WATER EFFLUENTS (SAMPLE #2)

Source of Sample	μ_{max}, day^{-1}; Sample Concentration, (%), and statistical Significance; N: Significant Difference at 95% Confidence Level
Raw Sewage	0.120 (1.0%) 0.078 (1.0%) 0.015 (100%)
Primary Effluent	0.107 (1.0%) 0.015 (1.0%) 0.014 (100%)
Secondary Effluent (A.S.)	0.078 (1.0%) 0.045 (1.0%) 0.012 (100%)
Tertiary Effluent	0.100 (1.0%) 0.041 (1.0%) 0.009 (100%)

TABLE 4-6

MAXIMUM GROWTH RATE, μ_{max}, CORRESPONDING SAMPLE CONCENTRATION, AND STATISTICAL SIGNIFICANCE OF RESULTS FROM DIFFERENT SAMPLE CONCENTRATIONS (%) FOR EFFLUENT WASTE WATER EFFLUENTS (SAMPLE #2)

Sample Concentration	μ_{max}, day^{-1}; Source of Sample and Statistical Significance; N: Significant Difference at 95% Confidence Level
0.5%	0.104 (I) 0.102 (R) 0.089 (III) 0.078 (II)
1%	0.087 (III) 0.078 (R) 0.067 (II) 0.015 (I)
10%	0.049 (III) 0.014 (I) 0.013 (R) 0.012 (II)

Code:

(R) Raw Sewage
(I) Primary Effluent
(II) Secondary Effluent
(III) Tertiary Effluent

Inspection of Tables 4-27, 4-29, and 4-31 reveals that at the 0.1 percent and 1.0 percent sample concentrations there was no significant difference in the biostimulatory properties of any of the four types of waste water effluents. At the 10 percent sample concentrations there was no significant difference between any of the four types of effluents when measured by the maximum growth rate, $\hat{\mu}_b$. When the growth response was measured by the maximum growth rate, $\hat{\mu}_{t\ell}$ (Table 4-31), however, the rate for the 10 percent sample concentration of secondary effluent was significantly greater than the growth rate for 10 percent raw and tertiary effluents, but did not differ with the primary effluent response. Differences in biostimulatory response were not detectable for the 10 percent sample concentrations of raw, primary and tertiary effluents.

Expressing the biostimulatory properties in terms of maximum cell concentrations, $\hat{X}_S$ (Table 4-29), did not reveal significant differences in response in the 0.1 percent and 1.0 percent sample concentrations of any of the four types of waste water effluent samples. At the 10 percent sample concentrations, $\hat{X}_S$ showed the secondary effluent as yielding a significantly different and higher response than any of the other assays at this concentration. Response in the tertiary and primary 10 percent assays did not differ, nor did primary and raw when expressed as $\hat{X}_S$.

Tables 4-32, 4-34, and 4-36 present an analysis of $\hat{\mu}_t$, $\hat{\mu}_{t\ell}$, and $\hat{X}_S$ observed for 0.1%, 1.0%, and 10% concentrations of four STPUD waste water samples collected on 15 January 1969 (Sample #C).

Tables 4-33, 4-35, and 4-37 present a similar summary and statistical significance comparison of the three concentrations assayed.

Table 4-32 shows that only the primary effluent assays yielded significantly different growth rates ($\hat{\mu}_t$) at all three sample concentrations. The growth rates, $\hat{\mu}_t$, for raw sewage, and secondary and tertiary effluents at 0.1 percent and 1.0 percent sample concentrations did not differ statistically. Tables 4-34 and 4-36 show the same assay data with the growth response expressed as the maximum cell concentration ($\hat{X}_S$) and growth rate ($\hat{\mu}_{t\ell}$), respectively. Both methods of expressing growth response show the same statistical results for all three sample concentrations for the various samples. Only the 0.1 percent and 1.0 percent sample concentrations of secondary and tertiary effluents did not differ significantly when the growth response was expressed as $\hat{X}_S$ and $\hat{\mu}_{t\ell}$.

The statistical comparison of the three samples assayed for each waste water effluent revealed that of the 10 percent concentrations only the 10 percent tertiary effluent assay showed a different growth rate when the response was expressed as $\hat{\mu}_b$ (Table 4-35), and this difference was marginal. The comparison made in Table 4-35 for $\hat{X}_S$ values show only the 10 percent sample concentration assays of secondary and tertiary effluents differing significantly, whereas, the comparison of $\hat{\mu}_{t\ell}$ values shows that at a 10 percent sample concentration all four waste water samples gave a significantly different growth response.

A collective examination of Tables 4-20 through 4-37 reveals that statistically significant differences are somewhat more easily detected with the flask assay when the growth response is expressed as $\hat{X}_S$ or $\hat{\mu}_{t\ell}$. It appears that both methods are about equal in showing differences in growth response, and both should be used in data interpretation. The fact that it is possible to detect differences in growth at various sample concentrations for the four types of samples, and yet difficult to detect differences in response for the four types of samples (raw sewage and primary, secondary and tertiary effluent) apparently indicates that a toxicity effect is present in sewage and sewage effluents regardless of the degree of treatment to which it has been subjected. This hypothesis is supported by the apparent lack of direct increase in growth rate with increasing sample (nutrient) concentration. According to theory (Chapter II) a direct increase would be expected at concentrations less than the nutrient excess level. Also, in support of the toxicity concept is the approximately equal growth response obtained for all types of samples, which is also contrary to the theory that response increases with the

nutrient concentration in the nutrient limiting range. As the degree of treatment is increased, a reduction in toxicity would be expected; therefore, a greater response could be obtained in the tertiary effluent assays than for the less refined effluents even though the nutrient concentration in the tertiary assay flasks would be much less than that in the other flasks. However, the possibility of the increased release of bound forms of nutrients plus the production of vitamins, growth factors (biostimulants in general) with increasing degrees of biological treatment cannot be neglected as a factor in increasing the apparent biostimulatory properties of treated effluents.

Pearson et al. [17] have shown a similar toxic effect for domestic sewage and treatment plant effluents in fish bioassay data. Several thousand bioassays were performed on sewage samples collected throughout the San Francisco Bay Area and the toxicity effect of sewage was quite pronounced. The assays included samples containing only domestic sewage and a mixture of domestic sewage and industrial wastes; and the toxicity was detectable in all assays. Therefore, the fact that STPUD sewage is principally domestic in origin would appear to have little bearing upon the above hypothesis that sewage could exhibit a toxic effect in the algal assay procedure.

Seasonal Variation

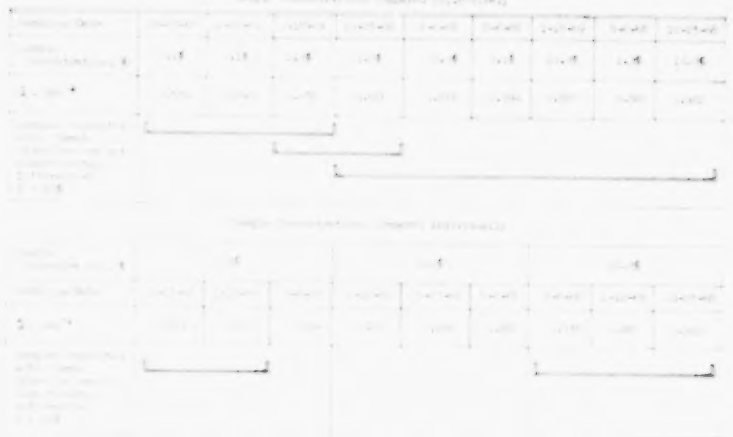
With the results of only three assays to compare it is difficult to make an analysis of seasonal variation in the waste water samples; however, several interesting comparisons are herein presented for each type of waste water assayed.

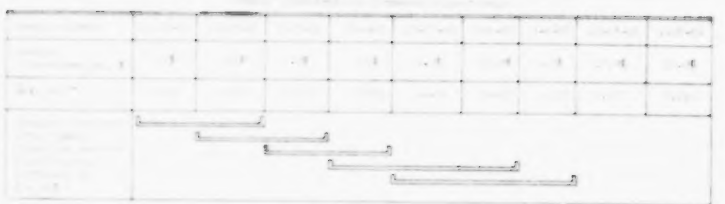
Raw Sewage. Tables 4-38, 4-39, and 4-40 present a comparison and statistical assessment of the maximum growth rates and cell concentrations observed at all three sample concentrations for the three raw sewage samples (6 August 1968, 24 October 1968, and 15 January 1969) assayed. When the values of $\hat{\mu}_t$ for all sample concentrations were compared collectively (Table 4-38), it was shown that the variation in the values of $\hat{\mu}_t$ within the five replicate assays were of such magnitude that statistically different response rates were difficult to detect. This is shown by the wide range of values of $\hat{\mu}_t$ underlined by the 95 percent significance bracket.

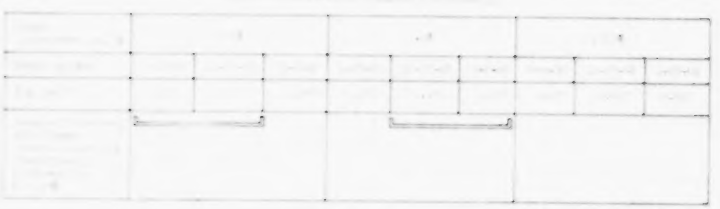
When values of $\hat{\mu}_t$ are compared individually (Table 4-38) the assays at the 0.1 percent sample concentration appeared to differ only in the sample collected on 6 August 1968. However, all three samples differed statistically at the 1.0 percent sample concentration, but neither was found to give statistically different growth rates at the 10 percent sample concentration. These results can be explained in part by the fact that the variances for the replicate assays differ considerably from one sample concentration to another and result in different statistical comparison levels. The decreasing growth rate with concentration increase (i.e., 1.0 to 10%) can also be explained by the apparent toxic effect exhibited by the sample of 6 August 1968, in which the response measured for this sample decreased at the 10 percent sample concentration.

Tables 4-39 and 4-40 show the same assay data for the raw sewage samples with the growth response expressed as $\hat{\mu}_{t,\ell}$ and $\hat{\lambda}_t$. Differences in growth response at the various sample concentrations are shown readily by both parameters, and both show essentially the same pattern for division. Examination of the rankings of the values of $\hat{\mu}_t$, $\hat{\mu}_{t,\ell}$, and $\hat{\lambda}_t$ reveals that $\hat{\mu}_{t,\ell}$ and $\hat{\lambda}_t$ are in the same order with the exception being the reversal of the values for the 1.0 percent and 10 percent sample concentrations of the 6 August 1968 sample; and that the ranking of the values of $\hat{\mu}_t$ differ from both $\hat{\mu}_{t,\ell}$ and $\hat{\lambda}_t$.

Primary Effluent. Tables 4-41, 4-42, and 4-43 present a comparison and statistical assessment of ranked means of the maximum growth rates and cell concentrations observed at all three sample concentrations for the three primary effluent samples analyzed with five replicate assays. Individual comparisons of the growth parameters are also shown in these tables. The collective comparisons of all concentrations again show that $\hat{\mu}_{t,\ell}$ and $\hat{\lambda}_t$ are superior to $\hat{\mu}_t$ in showing statistically significant differences in growth response. The $\hat{\mu}_{t,\ell}$ values showed a better division















in the ranked array than $\hat{\chi}_5$. The individual comparisons of growth parameter obtained at the three sample concentrations show that only the 6 August 1968 sample differed statistically at the 0.1 percent concentration regardless of the method used to express the growth response. At the 1.0 percent sample concentration $\hat{\mu}_{b\ell}$ and $\hat{\chi}_5$ yielded the same results, showing that samples collected on 6 August and 23 October 1968 do not differ statistically at the 95 percent confidence level, but that the 18 January 1969 sample response for primary effluent differs from the other samples. The $\hat{\mu}_b$ values show the 10 October and 18 January samples yielded results that do not differ at the 95 percent significance level.

Secondary Effluent. A comparison and statistical assessment of maximum growth rates and cell concentrations observed at all three sample concentrations for the three secondary effluent samples assayed are presented in Tables 4-44, 4-45, and 4-46. Individual comparisons of the growth parameters are also shown in these tables.

As shown for all previous samples, the values of $\hat{\mu}_{b\ell}$ and $\hat{\chi}_5$ show more distinct differences in growth response. All $\hat{\mu}_{b\ell}$ values differed at all concentrations of sample except for the 1.0 percent concentration of the samples collected on 6 August and 23 October.
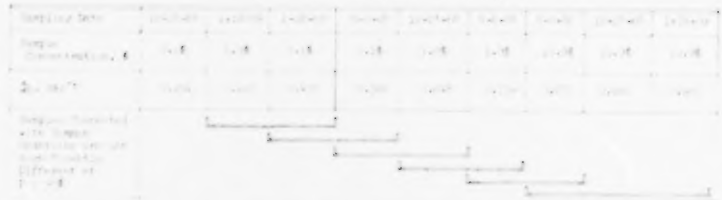
Waste water having undergone biological treatment processes (secondary effluent) might be expected to show less seasonal variation than raw or primary effluents provided the treatment plant is designed properly and not greatly underloaded or overloaded during any particular season of the year. The STPUD plant is more heavily loaded during the summer months because of the heavy influx of tourists and vacationers. However, the growth response expressed in terms of either of the three parameters does not show differences that appear to be related to the season of the year unless it is assumed that an increase in organic loading causes an increase in toxicity of the effluent. Such an effect could contribute to the growth response obtained for the 6 August sample, which was shown to be significantly less than the values for the other two samples at the 10 percent sample concentration when expressed as $\hat{\chi}_5$ or $\hat{\mu}_{b\ell}$, but gave the highest response at the 1.0 percent concentration.

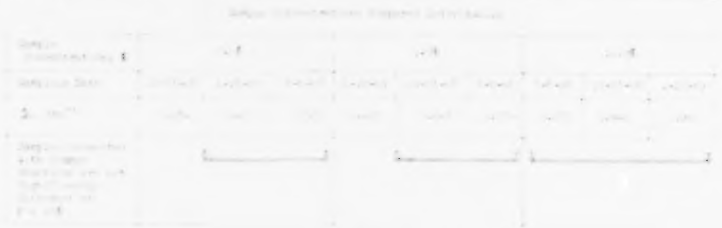
Tertiary Effluent. Tables 4-47, 4-48, and 4-49 show collective and individual comparisons and statistical assessment of the maximum growth rates and cell concentrations observed at the three sample concentrations for the three tertiary effluent samples assayed.

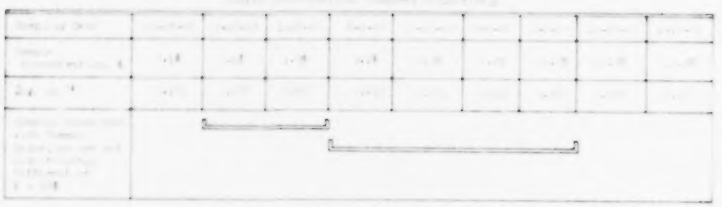
The collective comparisons of $\hat{\mu}_b$, $\hat{\mu}_{b\ell}$, and $\hat{\chi}_5$ show approximately the same number of values differing; however, the ranking of the various samples differs more in the tertiary effluent assays than any of the effluents previously discussed. In general, the sample concentration and sampling date relationships in raw, primary, and secondary effluents followed the same ranking, particularly in values of $\hat{\chi}_5$ and $\hat{\mu}_{b\ell}$, but for the tertiary effluent samples the order in which the values appear are varied for all three parameters. Perhaps a significant proportion of this disarray is attributable to the sample collected on 6 August. This sample contained a high concentration of chlorine which was not removed prior to the assays, thus inhibiting algal growth. The effect is quite pronounced in the 10 percent concentration assays. Also, a large response at the lower sample concentrations indicates that a significant improvement in response was obtained as the chlorine concentration was diluted.

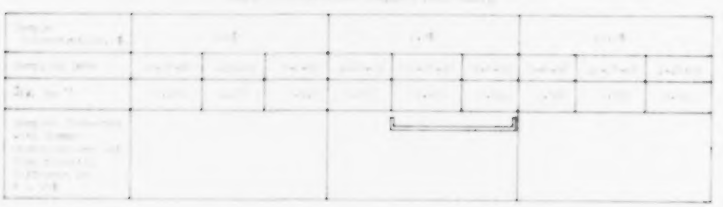
Evaluation of Waste Water Assays

In reviewing the results shown for all types of waste water treatment plant effluents, it is difficult to arrive at conclusions about seasonal variations when only three samples are available. However, from the limited data available it appears that $\hat{\mu}_{b\ell}$ and $\hat{\chi}_5$ yield more discernible results with which to compare algal growth response measurements. All types of waste water effluents apparently are toxic to algal growth in assay such as described in this chapter. Similar effects for waste water effluents have been reported in fish bioassay work. Further evidence of an apparent toxic effect in waste water effluents is shown in Tables 4-17, 4-18,

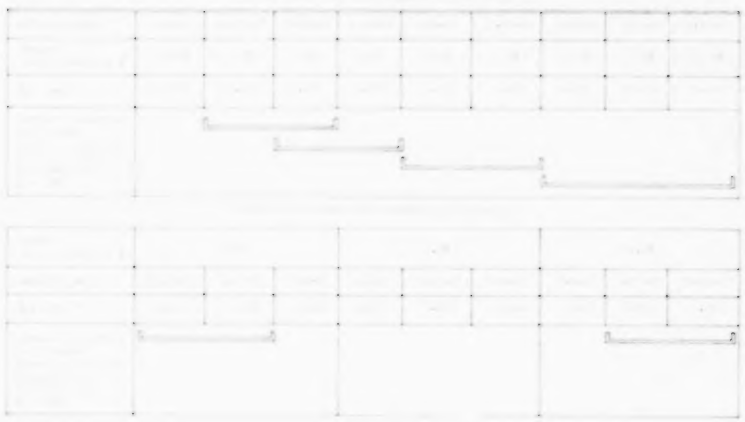












and 4-19 where response values for 50 percent sample concentration assay are reported for three samples other than the three discussed in detail above. There is a definite decrease in growth response for all three samples of secondary and tertiary effluents when the sample concentration is increased to 50 percent. Only one sample was assayed at the 50 percent concentration for raw sewage and primary effluent and there was no decrease in response in these samples. However, the secondary and tertiary effluent assays exhibited significant reductions in response when the sample concentration was increased from 10 to 50 percent. Although the raw and primary effluent assays did not show a decrease in response with an increase in sample concentration, a significant increase in growth rate did not accompany a substantial increase in nutrients.

Nutrient Concentration vs Growth Response

Chemical analyses, including concentration of nutrients, for the various waste water samples assayed are presented in Appendix B. Figures 4-7 and 4-8 show the observed variation in growth response ($\hat{\mu}_t$, $\hat{\mu}_{t\ell}$, and $\hat{\lambda}_s$) in relation to the concentration of total nitrogen and total phosphorus, respectively, (S_o) initially present in the flask culture using raw waste water and effluent samples from STPUD as the sources of nutrients.

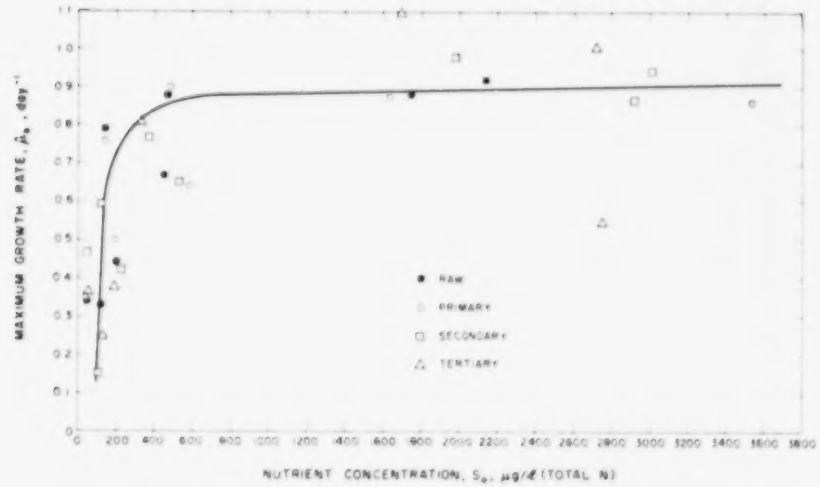
In Figure 4-7 the values of $\hat{\mu}_t$ and $\hat{\mu}_{t\ell}$ appear to vary linearly with the initial concentration, S_o, of total nitrogen up to about 500 $\mu g/\ell$. Using the analogy of the Michaelis-Menten nutrient concentration at half the maximum growth rate and as only the initial concentration value, S_o, was obtained in the flask assays, it appears as with the creek water assays, a reasonable estimate of K_{s_o} for S. gracile is about 150 $\mu g/\ell$ of total nitrogen. From Figure 4-8 it appears that in waste water effluents the concentration of initial phosphorus, S_o, beyond which S. gracile approaches a maximum rate of increase is approximately 50 $\mu g/\ell$. Only a 10 $\mu g/\ell$ concentration of total phosphorus was required for the organisms to approach the maximum growth rate. Thus it may be said from the two figures that a reasonable estimate of K_{s_o} is 150 $\mu g/\ell$ for nitrogen and 10 $\mu g/\ell$ for phosphorus.

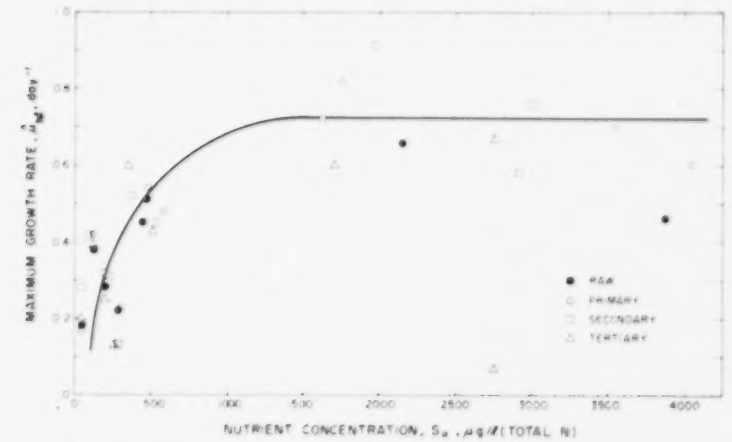
The variation of growth response, as measured by $\hat{\mu}_t$, $\hat{\mu}_{t\ell}$, and $\hat{\lambda}_s$, in relation to various forms of nitrogen and phosphorus and reactive iron is shown in Figures C-6 through C-15 (Appendix C). Figure C-14 yields an estimate of K_{s_o} for reactive iron of less than 2 $\mu g/\ell$ in contrast with the 5 $\mu g/\ell$ estimated from an analysis of creek water growth response data. From Appendix B it is evident that the concentration of iron was much higher in the surface waters than in the effluents, consequently the data from the creeks should indicate a higher estimate than those from the effluents unless reactive iron was the limiting factor in both cases. This indicates that iron was not in fact the rate limiting nutrient in the flask assays cited.

4. Experimental Ponds

During steady state operation of experimental ponds described in Chapter VI, samples for flask assay were collected from each of the eight ponds during two separate assays of LTW, and of secondary and tertiary waste water effluent. These flask assays were intended to study the variation in the residual growth stimulating ability of water which has previously supported a rapid growth of algae, presumably consuming most of the rate limiting nutrient in the process. Thus the flask cultures assayed a quite different medium than was originally introduced into the ponds — a situation analogous to that often encountered in the field when samples are collected from a natural lake for analysis following an algal bloom.

Results of the flask assay of 16 pond samples are summarized in Table 4-50, in which are reported values of growth rates, cell concentration, coefficient of variation for $\hat{\mu}_t$ and $\hat{\lambda}_s$, and the correlation coefficient for $\hat{\mu}_{t\ell}$. Values presented in the table for concentration of sample, of course, represent the influent waste concentration established for the pond assay and do not reflect the condition of the various effluents at the time samples were drawn from the pond for flask assay. The procedure was simply to inoculate five 150 $m\ell$ portions of a pond sample with S. gracile and to culture the flask for five days in the usual manner.





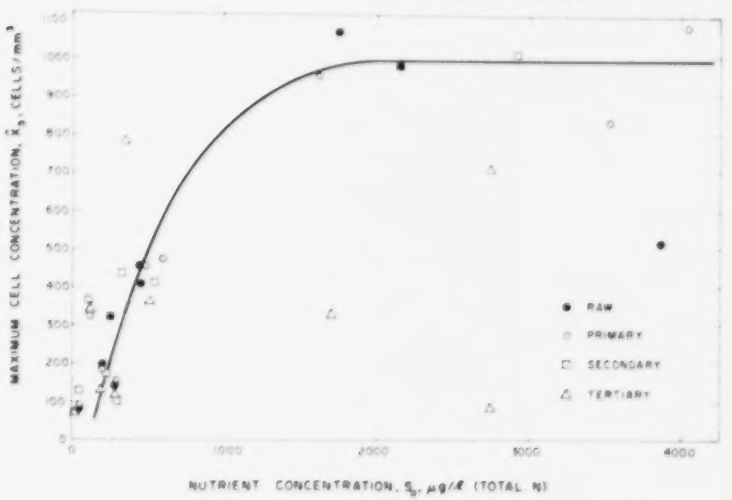
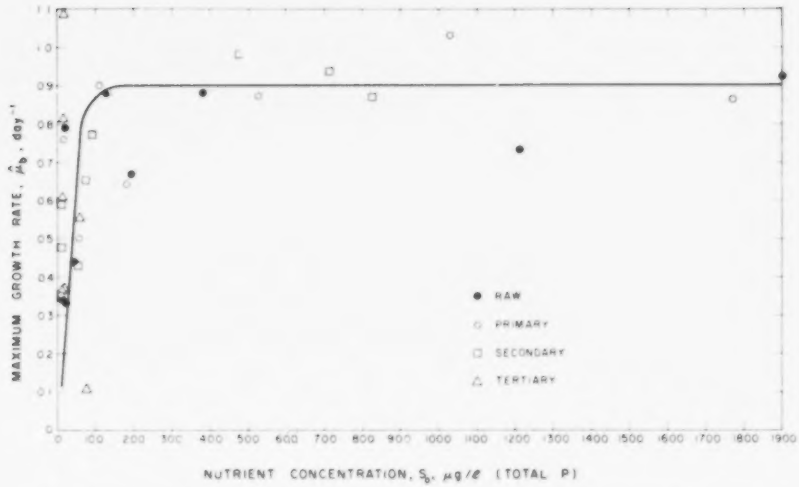
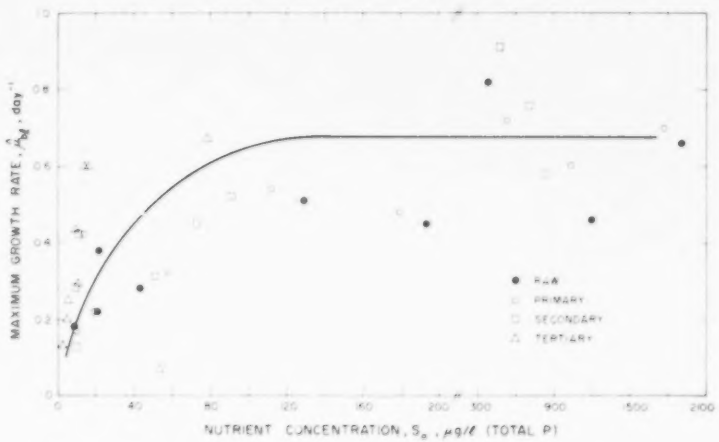


FIGURE 4-7 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_o$ AND $\hat{\mu}_d$, AND CELL CONCENTRATIONS, $\hat{X}_o$, WITH CONCENTRATION OF TOTAL NITROGEN IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM SIPUD AS THE SOURCE OF NUTRIENT





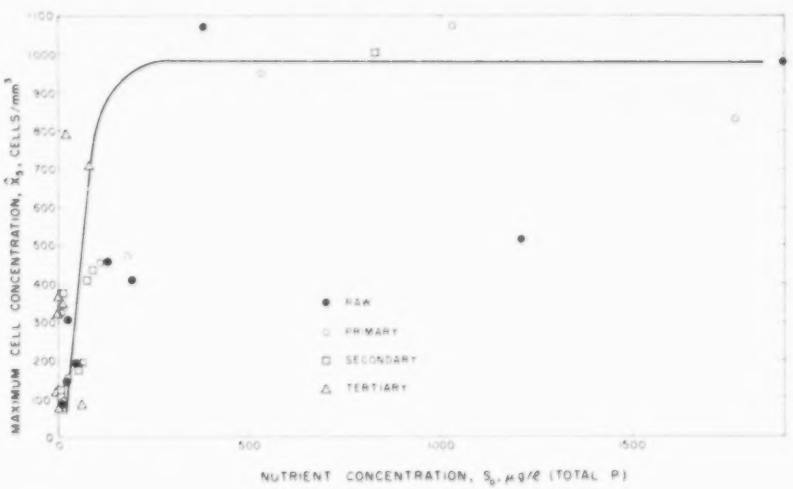


FIGURE 4-8 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_M$, AND CELL CONCENTRATION, $\hat{X}_3$, WITH CONCENTRATION OF TOTAL PHOSPHORUS IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT

TABLE 4-50

MAXIMUM GROWTH RATES, $\hat{\mu}_0$ and $\hat{\mu}_{0\ell}$, AND MAXIMUM CELL CONCENTRATION, $\hat{X}_5$, ATTAINED AT THE END OF FIVE DAYS IN FLASK CULTURE OF POND SAMPLES COLLECTED DURING STEADY STATE OPERATION

Sampling Date, Pond and Assay No.	Source and* Conc. of Sample (%)	Mean Max. Rate ($\hat{\mu}_0$, day^{-1})	Coef. Var. (%)	Mean Max. Rate ($\hat{\mu}_{0\ell}$, day^{-1})	Corr. Coef.	Cell Conc. $\hat{X}_5$ (cells/mm^3)	Coef. Var. (%)
10-4-68							
Assay #6							
Pond 1	1.0% II	0.418	24.1	0.336	0.9538	186.0	8.4
Pond 2	0.1% III	0.246	25.2	0.199	0.8967	101.8	5.8
Pond 3	1.0% III	0.248	33.9	0.231	0.9180	115.6	10.5
Pond 4	1.0% III	0.358	21.8	0.255	0.9403	121.6	9.7
Pond 5	LTW Control	0.196	63.5	0.207	0.8602	106.4	19.5
Pond 6	0.1% II	0.220	33.5	0.199	0.8372	100.4	11.5
Pond 7	1.0% II	0.330	48.7	0.262	0.8685	138.0	26.4
Pond 8	1.0% II	0.534	7.1	0.285	0.8161	140.2	26.3
11-1-68							
Assay #7							
Pond 1	LTW Control	0.380	58.7	0.344	0.9077	191.6	9.2
Pond 2	0.1% III	0.433	28.7	0.300	0.8708	162.5	19.3
Pond 3	1.0% III	0.408	46.7	0.329	0.8988	183.0	22.5
Pond 4	1.0% III	0.478	45.0	0.412	0.9224	278.8	48.1
Pond 5	LTW Control	0.290	44.6	0.315	0.8588	180.6	10.0
Pond 6	0.1% II	0.376	27.6	0.336	0.8640	188.8	7.2
Pond 7	1.0% II	0.340	19.2	0.344	0.8765	205.4	7.4
Pond 8	1.0% II	0.374	13.3	0.412	0.9392	294.2	12.3

*Code
II = Secondary Sewage Effluent
III = Tertiary Sewage Effluent
LTW = Lake Tahoe Water

As observed in all other flask assays previously reported in this chapter, assessment of growth response using $\hat{\lambda}_5$ data appears to be more precise than that measured by $\hat{\mu}_1$. The coefficients of variation for each sample assayed were smaller for the $\hat{\lambda}_5$ data in 14 of the 16 assays, and one of the two assays yielding higher coefficients of variation for $\hat{\lambda}_5$ did not differ significantly (99 percent level) from the value of $\hat{\mu}_1$.

Highly significant (99 percent confidence level) correlation coefficients were obtained for the least squares fit of the cell concentration data to the log growth equation for all 16 samples assayed.

Statistical Comparison. Tables 4-51, 4-52, and 4-53 present a comparison and statistical assessment of ranked means of $\hat{\mu}_1$, $\hat{\mu}_{1,\ell}$, and $\hat{\lambda}_5$ observed for all 16 pond samples assayed. Again, the variation in the values of $\hat{\mu}_1$ (Table 4-51) within the five replicate assays were of such magnitude that statistically different response rates were difficult to detect. Twelve of the 16 values are shown not to differ significantly.

Table 4-52 also shows that there were few differences in response in the flask assays of pond samples, as measured by $\hat{\mu}_{1,\ell}$. In this case the lack of detectable differences can be attributed to a similarity in sample characteristics, evidenced by smaller variation in correlation coefficients (Table 4-50). Such a finding is, of course, not surprising when it is considered that all of the samples came from ponds operating with a near constant mass of organisms (~ steady state) and a near constant concentration of nutrients.

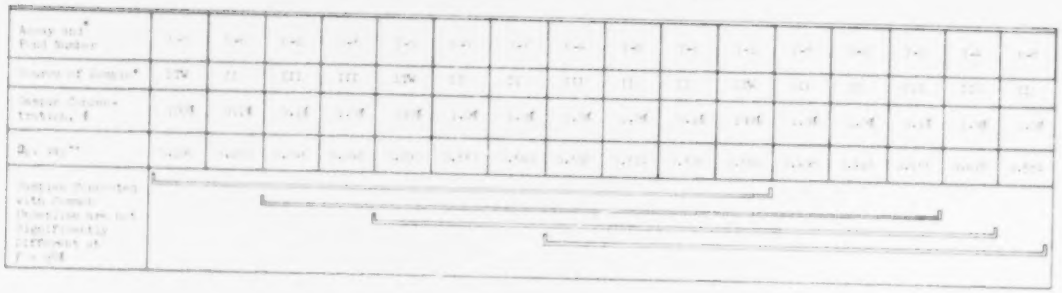
The lack of difference in response in the pond samples is again shown by the $\hat{\lambda}_5$ values in Table 4-53. Here the underline brackets show a division of five ranges of $\hat{\lambda}_5$ in contrast with four for $\hat{\mu}_1$ (Table 4-51) and only three for $\hat{\mu}_{1,\ell}$ (Table 4-52). However, one-half of the samples are shown not to differ in growth response.

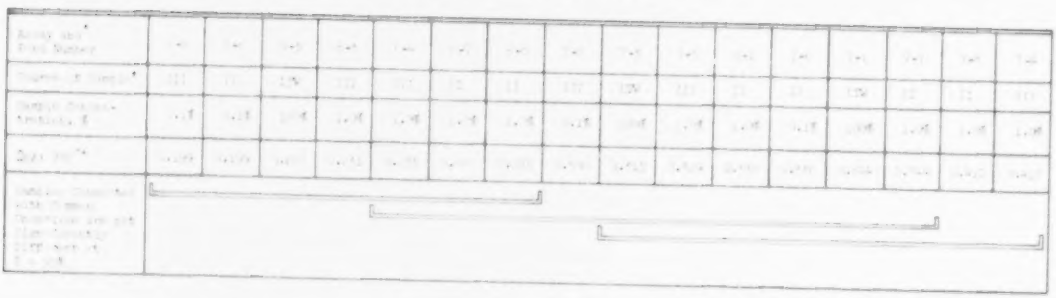
As noted for many of the other flask assays, the order in which the $\hat{\lambda}_5$ and the $\hat{\mu}_{1,\ell}$ values are ranked is essentially identical; whereas, the order in which the $\hat{\mu}_1$ values are ranked is completely different than either $\hat{\lambda}_5$ or $\hat{\mu}_{1,\ell}$. This difference appears to be caused primarily by the random variation in experimental error within each replicate assay. Specifically, the cell counts for determining the $\hat{\lambda}_5$ values involve less experimental error than the first and three-day counts because the much larger cell concentration on the fifth day allows a larger error in individual cell counting before the effect of the error is detectable. Similarly, the values of $\hat{\mu}_{1,\ell}$ are obtained from a least squares fit of cell count data; therefore, all of the individual errors are averaged and less variation appears within the response measurements.

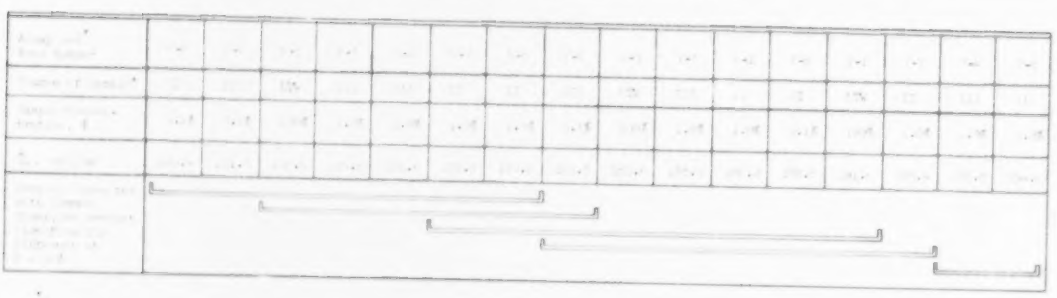
5. Indian Creek Reservoir

As a supplementary phase of the demonstration project herein reported, a program was initiated to monitor the growth stimulating potential of tertiary STPUD waste water effluent held in the Indian Creek Reservoir. The objective was to observe the history of a lake newly created with tertiary treated waste water; and to compile data useful in an estimate of the nutrient balance necessary to a scientific and engineering interpretation of the project's findings in terms of eutrophication of surface waters.

Sample collection and analysis commenced just prior to the winter season of 1968. Flask assays were made of reservoir water samples collected on 2 October and 4 November of that year. Table 4-54 shows the values of $\hat{\mu}_1$, $\hat{\mu}_{1,\ell}$, and $\hat{\lambda}_5$ reported for these assays, together with the corresponding coefficients of variation and correlation coefficients. Too few data are available for statistical comparison of the three measures of growth response of *S. gracile*. Nevertheless, some interesting preliminary observations can be made from a comparison of the results shown in Table 4-54 and those shown in Table 4-55, which summarizes the response observed in flask assays of STPUD tertiary effluent.







From Table 4-55 it appears that in the assay of 23 October the growth response increased with the concentration of effluent in LTW as that concentration was increased from 0.1 percent to 1.0 percent to 10 percent. The sample of 25 June shows little difference between the 1 percent and 10 percent values of $\hat{\mu}_t$, but a drastic decrease when a 50 percent concentration was assayed. Inspection of the chemical analysis data (Appendix B) reveals that the phosphorus content was less than 1 mg/ℓ on both sampling dates; and nitrogen was between 20 and 25 mg/ℓ. Thus N/P ratios were of the order of 27:1 in June and 35:1 in October. Data furnished by the STPUD for June 1968 shows similar values, although on 28 June the N/P ratio was 14.6:1. Therefore, it appears that the samples assayed were chemically within the range of values assumed to be phosphorus limiting, with nitrogen values not particularly different from sample to sample in Table 4-55. The evidence suggests that some significant toxic effect was critical when the concentration of tertiary effluent was increased from 10 to 50 percent — a conclusion supported by the results of other assays discussed previously in this chapter.

Values of $\hat{\mu}_t$ shown in Table 4-54 for 50 percent concentrations of reservoir water, which in October still remained essentially tertiary effluent, show a much higher growth response than reported for the June assay of freshly collected tertiary effluent. The cause of this higher response is not obvious. Chemical analyses (Appendix B) show that on 3 October 1968 the sample taken at the outlet of the reservoir had a nitrogen content of 18 mg/ℓ and a phosphorus content of .034 mg/ℓ. On 4 November 1968 the corresponding values were 13.1 mg/ℓ-N and 0.14 mg/ℓ-P. Thus apparent N/P ratios of 550:1 and 93:1 are indicated. Such phosphate limiting ratios suggest, as in the flask assays of pond samples, that the algal growth had already taken up much of the rate limiting nutrient before the samples were taken for assay. The magnitude of $\hat{\mu}_t$ values for reservoir samples (0.862 and 0.752) in comparison with the 0.49 value of Table 4-55 for the tertiary effluent, suggest also that the toxic factor had likewise been removed. Whether this was due to algal growth, oxidation or precipitation of the toxicant, or other unidentified process, is not known.

In evaluating the preliminary findings presented above it is particularly important to bear in mind that the reservoir water at the time of sampling represented tertiary treatment by phosphate removal only, and during a period when the phosphate removal process was reported to be not functioning at maximum efficiency. Consequently, the nutrient concentrations herein reported are not representative of the tertiary effluent being delivered to the reservoir in 1969. For example, the phosphorus content of STPUD effluent in March 1969 ranged from 0.10 to 0.51 mg/ℓ, with nitrogen at the same time ranging from 0.6 to 0.72 mg/ℓ.

4. Time, t_o, to Reach Maximum Growth Rate, $\hat{\mu}_t$

In preceding sections, growth rates have been considered from the standpoint of maximum slope of the log growth curve and maximum cell concentration achieved when the logarithmic phase of growth had ended. An additional parameter of the growth curve in flask bioassays is the time, t_o, required for the response of inoculum to nutrient to yield the maximum growth rate, $\hat{\mu}_t$. Because cell counts were made only at the end of 1, 3, and 5 days, the growth curve representing the ideal shown in Figure 2-2 (Chapter II) could be plotted with only 4 points. Thus the choice of slopes of the curve was confined to the time sectors 0-1, 1-3, and 3-5 days. t_o was then taken as the time represented by the midpoint of the line having the steepest slope.

In the First Progress Report [1] it was reported that the majority of all assays at all sample concentrations reached the maximum growth rate within three days from the start of assay. The same results were observed for the flask assays reported herein; and is the basis for adopting five days as the terminal time for flask assays. That little time is required for the organisms in the culture flask to approach the log phase of growth is readily shown by the correlation coefficients obtained from a least squares fit of all cell count data observed for each assay. In all cases the results showed the correlation coefficient to be significant at

probability levels greater than 99.9 percent, which indicates that the inoculum begins to grow rapidly upon being added to the assay flask; and maintains or quickly regains its log phase growth rate when transferred from the standard culture. Nevertheless, the limited data defining the growth curve and the vagaries of cell counting combine to make $\hat{\mu}_1$ the most variable of the measures used to assess growth response in the flask assays.

B. SOLIDS AND CELL MASS DETERMINATIONS

At the end of the fifth day of culture the contents of the five replicate flasks were mixed together and the suspended solids of the mixture determined. The results of such determinations for 67 assays are presented in Table 4-5, along with values of cell mass, cell concentrations, and growth rates.

Cell mass was determined by dividing the suspended solids by the cell concentration, on the assumption that the suspended solids were principally cells of S. gracile. Such calculations yielded values of mean cell mass that ranged from 20.8 to [illegible] μμg/cell, and averaged 39.2[illegible] μμg/cell with 95 percent confidence limits for the mean equaling 39.2[illegible] ± 2.8[illegible] μμg/cell. The coefficient of variation for all 67 mass determinations was 29.8 percent, which indicates that a wide variation from the mean existed. Because cell counts were made from each of the five replicate flasks but suspended solids measurements were made only on the mixture of the five, there are no replicate data from which to determine what proportion of the variation is attributable to experimental error. However, from the analysis of cell count and solids determination data, coefficients of variation of 6 and 10 percent, respectively, were shown when the counts and solids determinations were made under well controlled conditions. It is logical therefore to assume that a significant proportion of the 29.8 percent variation is attributable to experimental error.

Statistical Comparison. Table 4-5 shows a comparison of the mean cell mass values obtained for cultures of S. gracile in various concentrations of samples from various sources. Coefficients of variation are also shown in the table for each sample source. Statistical significance of the data at the 95 percent probability level is shown by the underline brackets. Because of the wide fluctuation of cell mass within each type of sample, as shown by the coefficients of variation, only the LTW was found to differ statistically from creek and waste water (sewage) in the mean cell mass produced.

TABLE 4-57

COEFFICIENT OF VARIATION, AND STATISTICAL SIGNIFICANCE
OF RESULTS OF CELL MASS DETERMINATION
FOR *S. gracile* IN VARIOUS MEDIA

Source of Sample	T-T	S	P	ME	I	W	LTW
Coefficient of Variation, %	25.7	21.2	15.0	23.9	42.1	34.5	28.3
Mean Cell Mass, μμg/cell	34.0	34.4	37.5	40.0	41.8	42.2	48.0
Samples Connected with Common Underline are not Significantly Different at P = 95%	[underline]		[underline]				

Code

T-T = Truckee-Trout Creek
S = Sewage
P = Pond Runs
ME = Modified Skulberg Medium
I = Incline Creek
W = Ward Creek
LTW = Lake Tahoe Water

Cell Mass vs Growth Rates. Observed data on cell mass and corresponding growth rates, μ_A and μ_g, are plotted in Figure 4-9 for flask assays of various nutrient sources. Herbert [18] has shown that the mean cell mass for various bacteria increases with a decrease in residence time when grown in continuous culture at steady state. However, in Figure 4-9 there is no identifiable pattern of relationship of cell mass to growth rates. There are probably many reasons for this difference between batch and continuous culture systems. A significant reason could be the decrease in nutrient concentration as the flask culture increases in number of cells, whereas in a continuous flow system the nutrient concentration is a constant during steady state conditions. The effect on growth of the accumulation of waste products and/or biostimulants in a flask culture could also be a factor in the cell mass-growth rate relationship.

Cell Mass vs Cell Concentration. Figure 4-10 shows the observed variation of cell concentration with the mean cell mass for various samples and concentrations. Although the data points are widely scattered, it appears that some basic pattern emerges. The cell mass appears to increase with a decrease in cell concentration, the increase being more at cell concentrations below 100 cells/mm^3. The lower cell concentrations reported generally occurred in assays of samples having low nutrient concentrations. Under such conditions, the cells might be expected to accumulate nutrients and substrate and to become much larger in mass before dividing because of the growth limiting conditions present in the material being assayed. Figure 4-10 indicates also that there is a minimum size for cells of *S. gracile* under the conditions observed. This minimum appears to be approximately 30 μμg/cell.

More data are needed before any of the foregoing observations can be confirmed, including replicate determinations sufficient to assess the experimental error.

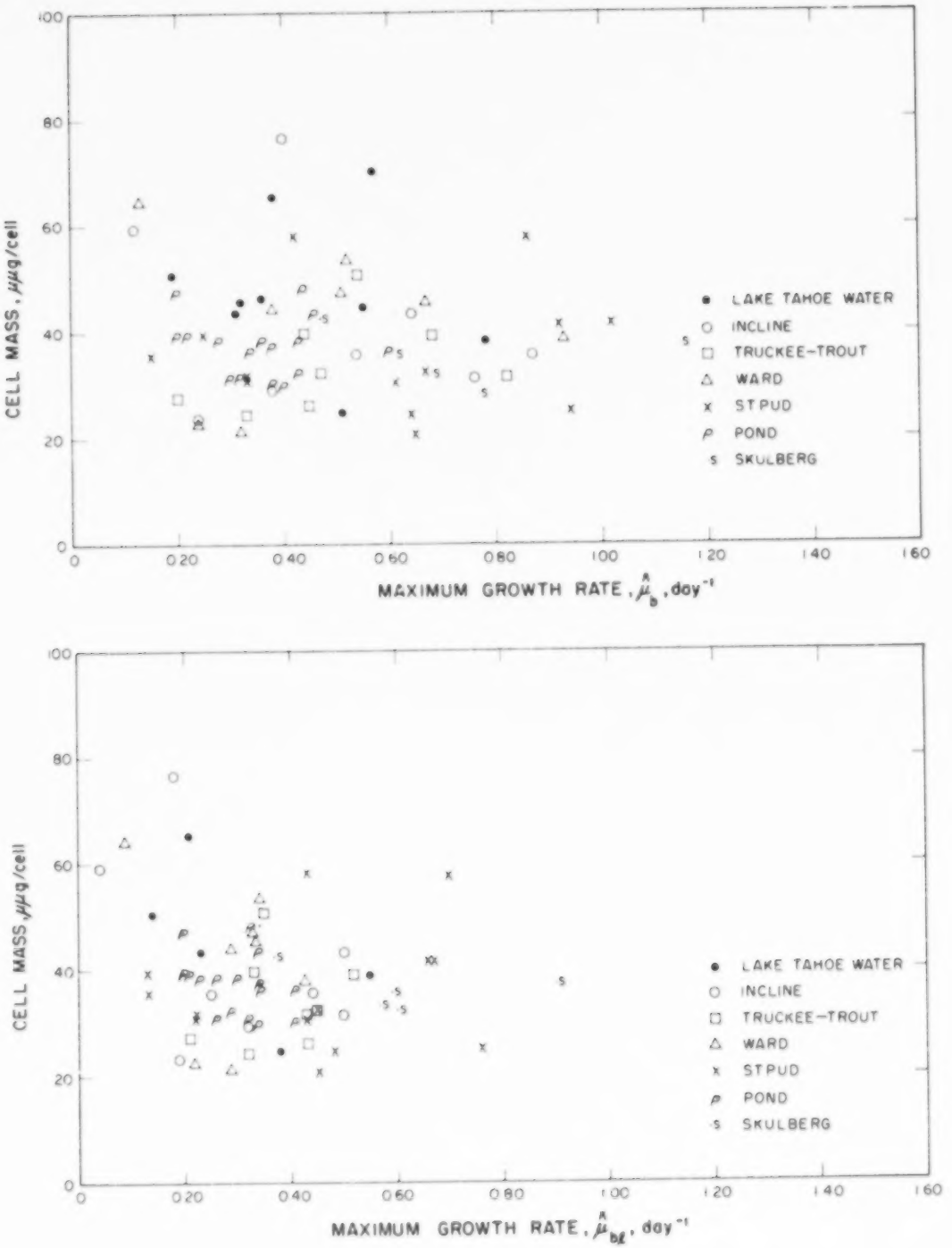


FIGURE 4-9 VARIATION OF CELL MASS WITH THE GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{b\ell}$, FOR S. gracile

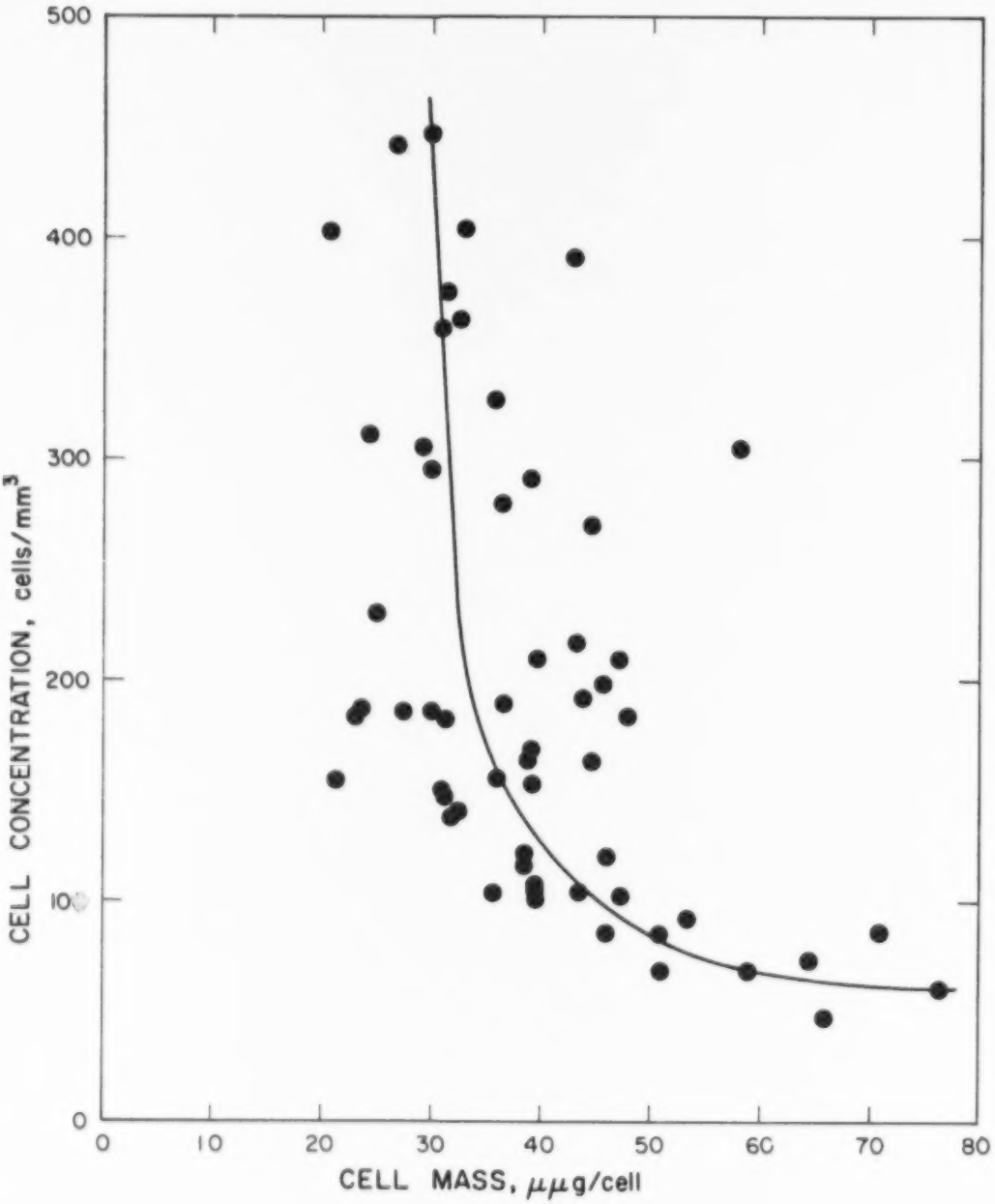


FIGURE 4-10 VARIATION OF CELL MASS WITH CELL CONCENTRATION

Nutrient Concentration vs Suspended Matter. Chemical analyses for all samples analyzed for suspended matter are presented in Appendix B. Variation of the concentration of suspended solids in relation to the concentration of various forms of nitrogen and phosphorus, and reactive iron, appear in Figures C-16, C-17, and C-18 of Appendix C. The most sharply defined relationship between suspended matter and nutrient concentration appears in the cases of ammonia nitrogen and of ammonia plus nitrite and nitrate nitrogen. (See Figure C-16.) The figure shows that for ammonia the relationship was essentially linear up to a concentration of 150 μg/ℓ NH_3-N. For the sum of ammonia, nitrite, and nitrate the linear relationship continued up to a concentration of 200 μg/ℓ.

Because suspended matter was principally algal cells it would be expected to show approximately the same relationship with nutrient concentration as with cell concentration, $\hat{X}_s$. Examination of Figures C-1 through C-15, reveals that such is essentially the case.

CHAPTER V

CHEMOSTAT (CSTR) ASSAYS

APPARATUS AND PROCEDURE

Chemostats of the type shown diagrammatically in Figure 5-1 were used for continuous flow assays. Each individual chemostat was cabinet-mounted and illuminated with 30-watt G. E. Coolwhite fluorescent lamps, No. 30T8-CW, providing 200-250 foot-candles (2150-2700 lux) light intensity. Two four-unit chemostat systems were set up. One of these (Figure 4-1, Chapter IV) was kept in the 20°C constant temperature room. The other was cooled by continuous flow of water through a central tube in each unit as shown in Figure 5-1. Aeration of each chemostat was accomplished by a small Dyna pump which discharged air through a sterile cotton-filled tube into the base of the chemostat at such a rate as to maintain a slow bubbling of air through the liquid. Agitation by this air served to keep the algae in suspension and aided in a rapid dispersion of the incoming medium, which was pumped into the chemostat by a small Sigmamotor pump. The overflowing effluent was collected in 500 ml Erlenmeyer flasks.

The operating procedure was to inoculate each chemostat with the test alga at a concentration level of 20-30 cells/mm^3, after first filling it with the test sample to be assayed. Thereafter the chemostat was fed with the sample at a continuous rate sufficient to provide the desired residence time (normally five days) and cell concentration was determined by cell counts at two-day intervals. The test was continued for at least two residence times after an apparent steady state was reached. For purpose of the study a steady state was assumed to prevail when cell counts for three successive periods checked within ± 20 percent, with no indicated trend. To develop data for kinetic constant evaluation, needed for interpretation of the results, at least three different residence times, θ, were made within the 3-15 day range.

Calculation of the growth rate of algae was made from cell counts and mass measurements as described in Chapter IV.

Eighty assays were made of samples from various nutrient sources diluted with Lake Tahoe water to yield concentrations adopted in the experimental design. In addition, four assays were made of Lake Tahoe water at various seasons. The general type of sample and the number of assays completed of each type are shown in the following table:

Type of Sample	Number of Bioassays
Surface runoff	9
Seepage	4
Sewage effluents	44
Chemical nutrients (N and P)	23
Lake Tahoe water	4
	84

Results of these assays were summarized as hereinafter described and interpreted in several contexts, including:

1. The relative ability of batch and continuous flow (chemostat) techniques to identify and quantify the growth stimulating potential of any source of nitrogen and phosphorous.

2. The limitations of the continuous flow method at its present state of development.

3. Kinetic theory (Michaelis-Menten)

4. The comparative influence of sewage effluents with and without nitrogen and phosphorous content to stimulate algal growth.

5. The source and concentration levels of nutrients which might cause significant eutrophication of Lake Tahoe.

In interpreting the results in relation to nitrogen and phosphorous the following assumptions were made as the basis for the Michaelis-Menten kinetic analysis.

1. Nitrogen (NO_3 + NH_3) is a rate limiting nutrient when the N/P ratio is less than 10.

2. Phosphorous (PO_4) is a rate limiting nutrient when the N/P ratio is greater than 15.

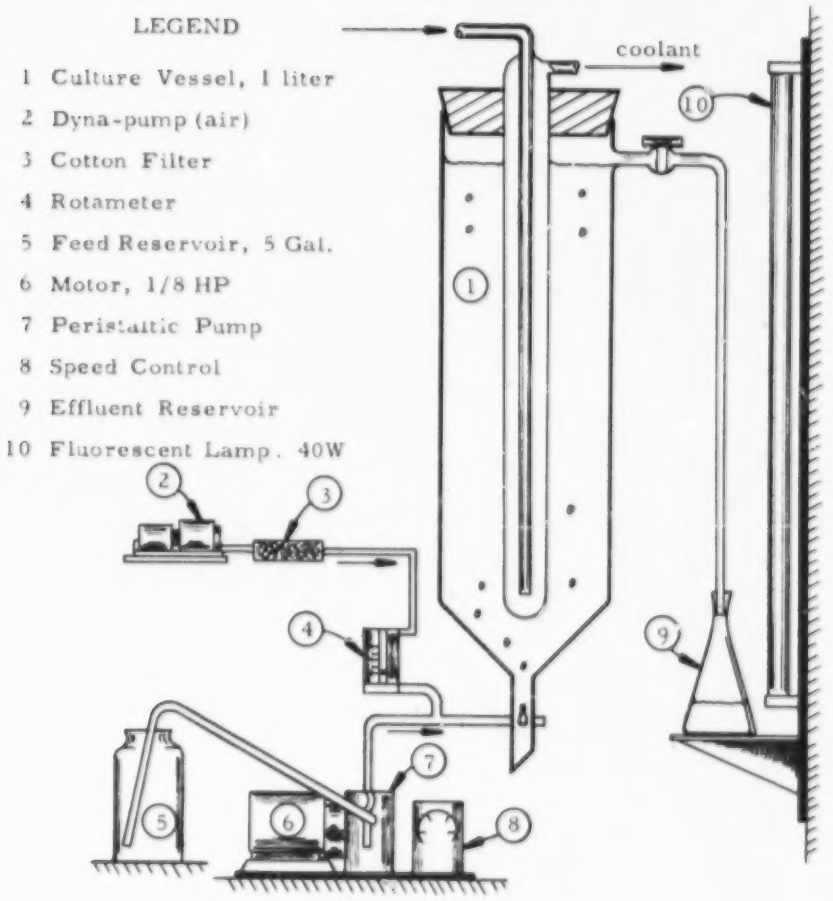


FIGURE 5-1 SCHEMATIC DIAGRAM OF CHEMOSTAT ASSAY SYSTEM

Available equipment and the time required to conduct steady state growth experiments prevented the conduct of parallel flask and chemostat assays of each sample collected. Consequently, as shown in the preceding table, the chemostat studies were largely concerned with sewage effluents and chemical nutrients. Only these two sources of nutrients were assayed in sufficient replications to warrant interpretations. Both improved techniques and additional replicate assays are required before reasonably valid interpretations can be made for the surface runoff and seepage sources on the basis of chemostat data. In these cases principal reliance must presently be placed on the flask assays reported in Chapter IV; and on pond assays scheduled for the future. Therefore, only sewage effluents, chemical nutrients, and Lake Tahoe water are discussed in this chapter.

SUMMARY OF DATA

Mean values of the results of physical and chemical analyses of sewage effluents and Lake Tahoe water are presented in Table 5-1.

The data obtained from the eighty-four continuous culture assays are summarized in Table 5-2, which reports the source concentration of the samples assayed; the steady state cell concentration, X_1, the dilution rate, D (μ); the concentration of nitrogen and phosphorus in the influent, S_o, and the effluent, S_1; and the hydraulic residence time, θ.

STEADY STATE CONSIDERATIONS

One of the problems encountered in the chemostat studies was the difficulty in achieving and maintaining steady state conditions. Although the chemostat concept is not new, dating back nearly half a century in its application to bacterial growth systems and some 25 years to the growing of algae, attempts to exploit the technique in the quantitative assay of the algal growth stimulating potential of waste effluents are of quite recent origin. Consequently, no standard procedure has been developed and there exist no operational parameters, such as define more established analytical procedures, by which to insure a definitive analysis. In this situation the dilution rate (residence time) was adjusted to some predetermined level which would permit microorganisms to achieve and maintain a constant biomass. With such uncertainty of test design it is not surprising that the bioassays were not uniformly successful in achieving steady state conditions.

Figures 5-2, 5-3, and 5-4, reproduced from the First Progress Report [1] are illustrative of the variation in results.

TABLE 5-1

SUMMARY OF MEAN PHYSICAL AND CHEMICAL ANALYSES
OF LAKE TAHOE WATER AND SEWAGE EFFLUENTS

CHARACTERISTIC	SAMPLE						
	Mid LTW	STPUD Raw	STPUD Prim.	STPUD Sec.	STPUD Tert.	Reno Prim.	Reno Sec.
pH	7.8	7.7	7.7	7.9	7.9	7.5	8.1
Conductivity, μmho/cm	83	500	435	454	610	400	450
Alkalinity, mg/l, $CaCO_3$	41	148	198	185	250	146	120
Chloride, mg/l, Cl	0.2	18	30	21.8	30	34	24
Iron, μg/l, Fe	10	380	200	12	10	10	25
NO_3, μg/l, N	5	1	23	680	2,000	1	1,390
NO_2, μg/l, N	1	19	10	1,320	14	19	460
NH_3, μg/l, N	60	29,270	29,100	23,250	18,750	21,500	12,000
Sol. organic Nitrogen, μg/l, N	95	2,900	1,800	1,400	1,040	1,500	350
Sol. inorganic (PO_4) Phosphorus, μg/l, P	5	9,220	5,250	7,400	700	9,200	9,000
Sol. organic Phosphorus, μg/l, P	5	3,800	2,950	1,020	25	2,700	1,000

TABLE 5-2

SUMMARY OF CHEMOSTAT EXPERIMENTS

Assay No.	Source of Sample	Conc. of Sample, %	S_1 cells/mm^3	D day^{-1}	S_0 µg NO_3-N/l	S_1 µg NO_3-N/l	S_0 µg NH_3-N/l	S_1 µg NH_3-N/l	S_0 µg PO_4-P/l	S_1 µg PO_4-P/l	g days
	(Sewage Effluents)										
1-3	Incline secondary effluent	1	70	0.100	245	7.5	20.5	1	178	36	10.0
1-4	Incline secondary effluent	10	420	0.102	2428	9.5	205	1	1760	60	9.80
2-3	(TC) primary effluent	10	700	0.188	[illegible]	[illegible]	4100	620	2800	1000	5.32
3-1	(TC) raw sewage	10	1500	0.189	1	5	2420	290	638	282	5.30
3-2	(TC) primary effluent	5	600	0.211	4	1	2080	98	900	1	4.74
3-3	Reno activated sludge effluent	10	1000	0.235	3.6	5	950	226	803	1	4.25
3-4	(TC) trickling filter effluent	10	1100	0.236	1.5	1	880	102	875	42	4.23
4-1	Standard rate oxidation pond effluent (American Canyon)	10	70	0.159	1.2	6	7	41	450	125	6.29
4-2	High rate oxidation pond (SERL, U.C.)	10	100	0.174	1	5	35	41	250	1	5.75
4-5	Reno activated sludge effluent	5	700	0.133	93	10	600	10	650	1	7.51
4-6	Activated sludge effluent (SERL, U.C.)	10	600	0.158	1500	10	10	5	1000	600	6.33
6-3	Reno activated sludge effluent	1	125	0.135	1	1	250	70	320	1	7.40
6-7	Reno activated sludge effluent	1	25	0.150	1	1	250	30	320	18	6.70
7-1	Reno activated sludge effluent	1	200	0.100	1	7.8	210	154	336	62	10.00
7-2	Reno activated sludge effluent	1	200	0.096	1	6.9	210	160	336	52	10.40
7-3	Reno primary effluent	1	-	0.143	1	6.8	215	258	302	42	7.00
7-4	Reno primary effluent	1	70	0.169	1	7.5	215	138	302	67	5.90
7-5	Reno activated sludge effluent	10	400	0.100	1	6.3	2100	145	3360	61	10.00
7-6	Reno activated sludge effluent	10	1400	0.120	1	3.7	2100	193	3360	30	8.30
7-7	Reno primary effluent	10	-	0.090	2	7.0	2150	9	3020	17	11.10
7-8	Reno primary effluent	10	700	0.115	2	9.8	2150	154	3020	29	8.70

(continued)

TABLE 5-2. --continued.

Assay No.	Source of Sample	Conc. of Sample, %	X_1 cells/mm^3	D day^{-1}	S_o µg NO_3-N/l	S_1 µg NO_3-N/l	S_o µg NH_3-N/l	S_1 µg NH_3-N/l	S_o µg PO_4-P/l	S_1 µg PO_4-P/l	θ days
9-1	Lake Tahoe Water	-	130	0.25	-	37	-		5	14	4
9-2	STPUD secondary effluent	10	260	0.25	760	180	280	"	2820	17	4
9-3	STPUD tertiary effluent	10	220	0.25	720	130	640	"	218	18	4
9-4	N & P @ 9-3 level	-	200	0.25	720	90	640	"	210	20	4
9-5	Lake Tahoe Water	-	124	0.25	-	23	-	"	-	10	4
9-6	N & P @ STPUD secondary 10% level	-	260	0.25	760	65	280	"	2820	"	4
9-7	N @ STPUD secondary 10% level	-	450	0.25	760	440	280	"	1	77	4
9-8	N @ STPUD secondary 10% level	-	245	0.25	720	200	640	"	1	16	4
10-1	Lake Tahoe Water	-	120	0.10	-	5	-	-	-	56	10
10-2	STPUD secondary effluent	10	175	0.06	650	2	400	435	620	44	15
10-3	STPUD tertiary effluent	10	150	0.06	740	350	970	435	180	23	15
10-4	STPUD tertiary effluent	10	150	0.10	740	5	970	400	180	12	10
10-5	N & P @ STPUD tertiary 10% level	-	(270)	0.06	740	3	970	400	180	"	15
10-6	N & P @ STPUD tertiary 10% level	-	210	0.06	740	10	970	400	180	185	15
10-7	N & P @ STPUD tertiary 10% level	-	(225)	0.10	740	2	970	400	180	22	10
10-8	N @ STPUD tertiary 10% level	-	185	0.10	740	2	970	350	-	90	10
11-1	Reno secondary effluent	10	1070	0.33	12	6	1680	250	2040	30	3
11-2	Reno secondary effluent	10	1000	0.33	"	1	"	200	"	110	3
11-3	Reno secondary effluent	10	1325	0.17	"	5	"	230	"	5	6
11-4	Reno secondary effluent	10	1000	0.17	"	5	"	235	"	30	6
11-5	Reno secondary effluent	10	700	0.11	"	5	"	48	"	50	9
11-6	Reno secondary effluent	10	840	0.11	"	5	"	120	"	45	9
12-3	Reno secondary effluent	1	90	0.33	1	5	168	50	204	5	3.0
12-4	Reno secondary effluent	1	90	0.33	1	5	"	38	"	5	3.0
12-5	Reno secondary effluent	1	160	0.17	1	10	"	100	"	10	6.0
12-6	Reno secondary effluent	1	175	0.17	1	10	"	50	"	8	6.0
12-7	Reno secondary effluent	1	70	0.11	1	5	"	180	"	5	9.0
12-8	Reno secondary effluent	1	65	0.11	1	5	"	150	"	5	9.0

*Data lost due to contaminated sample

TABLE 5-2. --continued.

Assay No.	Source of Sample	Conc. of Sample, %	X_1 cells/ml	D day^{-1}	S_0 µg NO_3-N/l	S_1 µg NO_3-N/l	S_0 µg NH_3-N/l	S_1 µg NH_3-N/l	S_0 µg PO_4-P/l	S_1 µg PO_4-P/l	θ days
	(Inorganic Chemical Nutrients)										
1-5	N and P @ 10% Incline secondary effluent level (50% flow)		1500	0.096	1214	110	1031	1	880	1000	10.40
1-6	N and P @ 1% Incline secondary effluent level		500	0.083	243	14	20.5	1	176	92	12.50
1-7	N @ 1% Incline secondary effluent level		75	0.099	243	10	20.5	1	-	16	10.50
2-5	NH_3 and P @ 10% TC primary effluent level		175	0.207	-	0	2300	2420	1700	1100	4.83
2-6	NO_3-N - 1000 µg/l		1150	0.187	1000	750	-	188	0	0	5.34
2-7	NH_3-N - 1000 µg/l		300	0.195	-	20	1000	800	0	0	5.13
3-7	NO_3-N - 500 µg/l		200	0.193	-	150	500	34	0	0	5.19
4-3	N and P @ 10% high rate oxidation pond effluent level		110	0.183	1	8	35	17	250	310	5.44
4-7	N and P @ 5% Reno activated sludge effluent level		90	0.133	70	20	600	20	70	65	7.63
5-1	NO_3-N - 250 µg/l: PO_4-P - 50 µg/l		125	0.180	250	15	0	0	50	1	5.55
5-2	NO_3-N - 250 µg/l: PO_4-P - 50 µg/l		150	0.190	250	15	0	0	50	1	5.31
5-3	NO_3-N - 500 µg/l: PO_4-P - 100 µg/l		200	0.170	500	16	0	0	100	1	5.81
5-4	NO_3-N - 500 µg/l: PO_4-P - 100 µg/l		350	0.190	500	15	0	0	100	1	5.38
6-4	N and P @ 1% Reno activated sludge effluent level		200	0.140	1	1	250	70	1	1	7.15
6-8	N and P @ 1% Reno activated sludge effluent level		25	0.150	1	1	250	30	320	18	6.70
6-5	Lake Tahoe Water		50	0.137	0	0	0	0	0	0	7.30
7-1	Reno secondary effluent	1	200	0.100	1	8	210	154	336	62	10.00
7-2	Reno secondary effluent	1	200	0.096	1	7	210	160	336	82	10.40
7-4	Reno primary effluent	1	70	0.170	1	8	215	138	302	67	5.90
7-5	Reno secondary effluent	10	400	0.100	1	6	2100	145	3360	61	10.00
7-6	Reno secondary effluent	10	1400	0.130	1	4	2100	143	3360	30	8.30
7-8	Reno primary effluent	10	700	0.114	2	10	2150	154	3020	29	8.70

(continued)

In Figure 5-2 are plotted a series of curves which, with one exception, show reasonable steady state growth after an initial period of rapid increase in cell count. In Figure 5-3 the reverse is essentially true, i.e., most of the curves show a decline in cell count after an initial increase. Figure 5-4 shows that in some of the less successful assays the decline in cell count was quite certainly due to "washout" of cells from the system. It might therefore be postulated that the decline in cell concentration in assays such as illustrated in Figure 5-3 was owing to a combination of loss of cells from the system as a result of inappropriate dilution rate and to other factors which may make the variation from steady state more apparent than real. These "other factors" include the reliability of cell count data from which the curves were established.

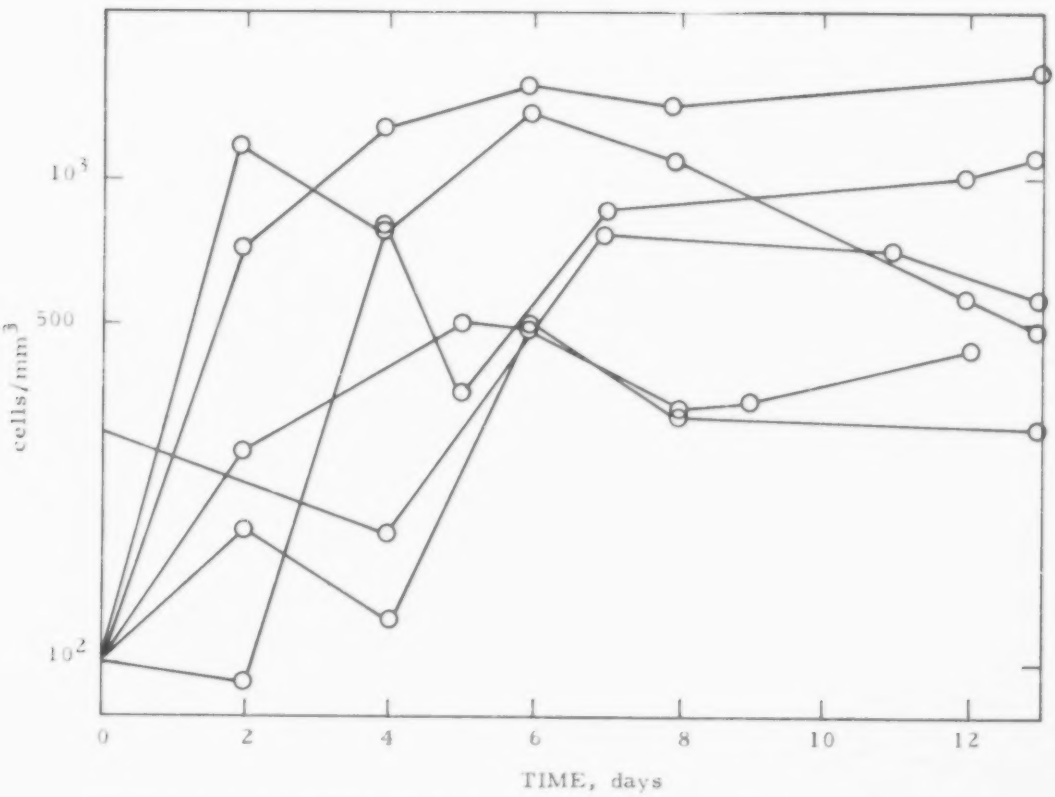


FIGURE 5-2 CHANGES IN ALGAL CELL CONCENTRATION WITH TIME FOR SELECTED WASTE WATER BIOASSAYS

As discussed in Chapter IV, one of the weaknesses of the assay techniques utilized is the accuracy with which cell concentration could be determined. In the chemostat assays this is critical because there is no accumulation of cell mass with time when a steady state is reached. Therefore, in assaying samples in which the rate limiting nutrient is present only in small amounts, the cell concentration at steady state must be small because of the nutrient-poor medium in which the alga was grown. The probability that any cell count will reflect the actual number of cells produced must then likewise be small, leading to fluctuations in cell count data and erratic behavior of plotted values. A similar problem, although one not reflected in the steady state consideration, can be expected in the accuracy with which changes in a small nutrient concentration which supports only a limited cell mass can be determined analytically.

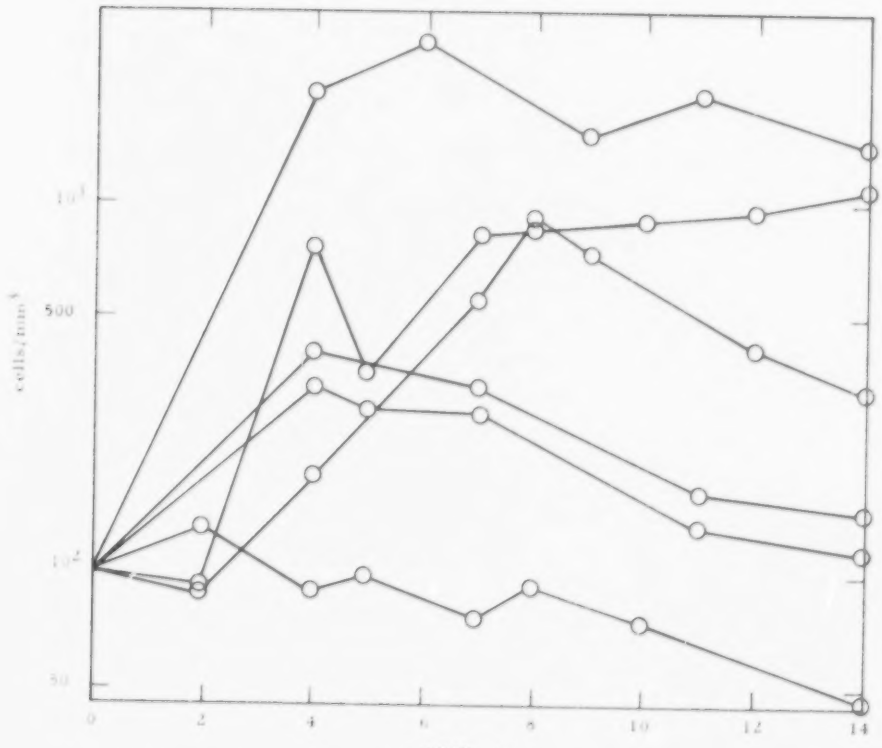


FIGURE 5-3 CHANGES IN ALGAL CELL CONCENTRATION WITH TIME FOR SELECTED WASTE WATER BIOASSAYS

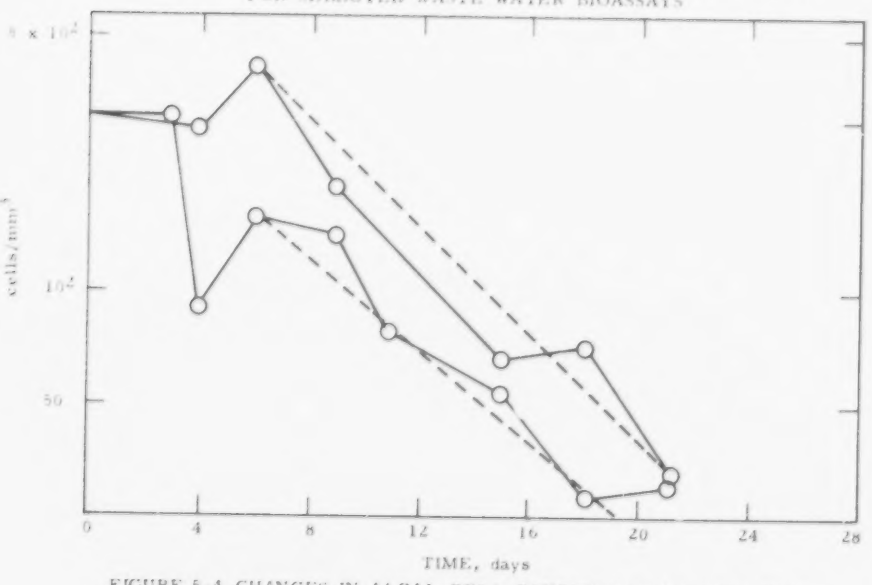


FIGURE 5-4 CHANGES IN ALGAL CELL CONCENTRATION WITH TIME FOR SELECTED WASTE WATER BIOASSAYS

The problems of achieving and identifying steady state conditions underscore the need for a standard chemostat procedure as well as the importance of replicate or multiple assays of any individual source of nutrients. However, where a significant number of assays were conducted, the chemostat studies yielded data which could be interpreted in the context of eutrophication of surface waters and the kinetics of growth stimulation by nutrients, as hereinafter discussed.

Figures 5-5 and 5-6 are examples of the results attained with sewage effluents during the period covered by this report. From the first of these two figures it may be noted that secondary effluent from the Reno treatment plant, at 10% concentration, achieved an apparent steady state cell concentration only after about 14 days at a residence time, θ, of 6 days. In contrast, the STPUD secondary effluent (Figure 5-6) at the same concentration, reached steady state after 2 days; however at a value of θ of only 4 days.

At 1% concentration, Reno secondary effluent did not reach steady state conditions but showed a continuous decline in cell concentration with time.

The steady state cell concentrations for Reno secondary effluents were four times those for STPUD secondary effluents. The nutrients supporting this growth differed only in nitrogen concentration; Reno secondary effluents being nearly twice that in the STPUD while the phosphorus concentration did not differ significantly in the two.

The growth responses of secondary and tertiary effluents of STPUD were essentially identical although the phosphorus concentration of secondary effluent was ten times that of tertiary effluents. Thus, it could be concluded that nitrogen in this culture was the limiting nutrient in the sewage effluents of STPUD.

A distinct difference between the growth response of LTW and 1% Reno sewage effluent appears in Figure 5-4, although both curves suggest failure of the assay through a loss of cells. However, if two residence times at approximately constant cell concentration are taken as defining steady state (Chapter III), the LTW assay was successful whereas the 1% Reno secondary effluent assay failed. Inasmuch as the difference in concentration of nutrients in the two samples was not significant, both being approximately the same as LTW, nutrient concentration alone cannot account for the observed results. The principal difference between the two bioassays was time of residence in the chemostat (θ = 4 days for LTW and 9 days for Reno effluent). Therefore, an explanation may be sought in the phenomena associated with dilution rate, or in some toxic factor present in the effluent.

In Figure 5-7 are plotted data from all bioassays completed for 10% and 1% sewage effluents in the study period. Theoretically, these curves should be horizontal lines, dropping off at the right-end as the dilution rate increases, i.e., as the days of residence, θ, become so small that algal cells and nutrient alike are passed through the chemostat too rapidly for growth to take place in the system. Similarly, at the left-hand side of the graph where the dilution rate is zero and the residence time infinitely long, the curves should drop off to some concentration typical of a flask culture of infinite duration. Exactly where the cell concentration begins to decline with time of residence (reduction in dilution rate) cannot be predicted theoretically. Reduced nutrient concentrations as well as toxic products of metabolism (Maček and Fencl [19]) produce this effect. Figure 5-7, however, shows that the breakpoint in the curves for both the 10% and 1% sewage effluents occurred at a dilution rate of about 0.13 days^{-1}, or at a residence time of about 8 days. The rate of decline differed somewhat, being more abrupt at the lower nutrient concentration, as might be expected on the basis of nutrient depletion.

From untreated LTW vs 1% Reno secondary effluent it is evident from Figure 5-7 that at a residence time of 9 days (dilution rate = 0.11 day^{-1}) the cell concentration produced by 1% Reno effluent lies in a range where the greatest deviation from the theoretical horizontal curve occurs. However, at a 4-day residence time (dilution rate = 0.25 day^{-1}) the observed data follow the generally horizontal curve. In the absence of a significant difference between nutrients in the LTW and Reno bioassays, it might be expected that at 4 days the two would be in agreement; and there is no evidence that such is not the case.

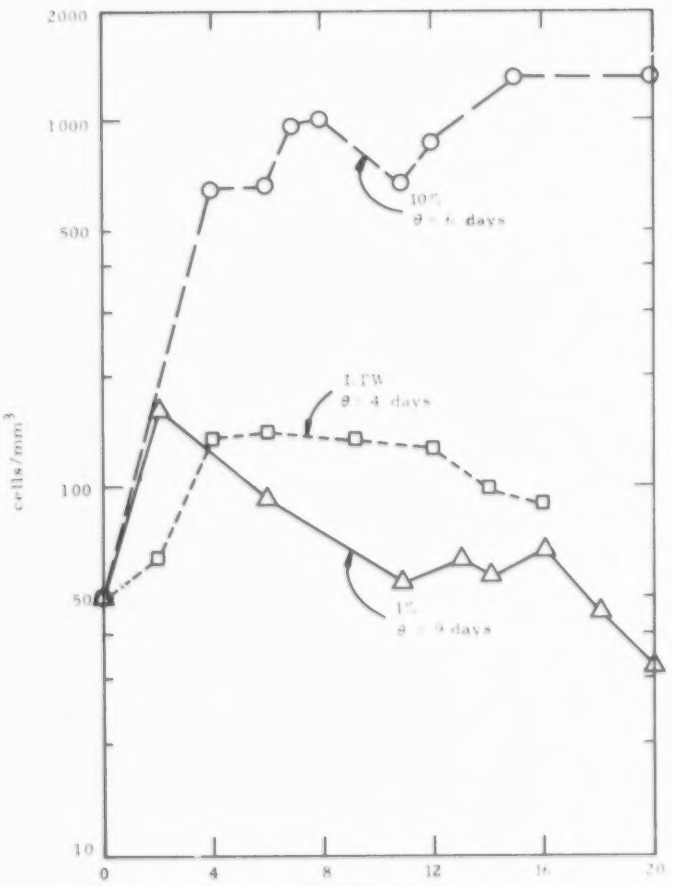


FIGURE 5-5 CELL CONCENTRATION WITH TIME FOR 1% AND 10% RENO SECONDARY EFFLUENTS IN CHEMOSTAT CULTURES

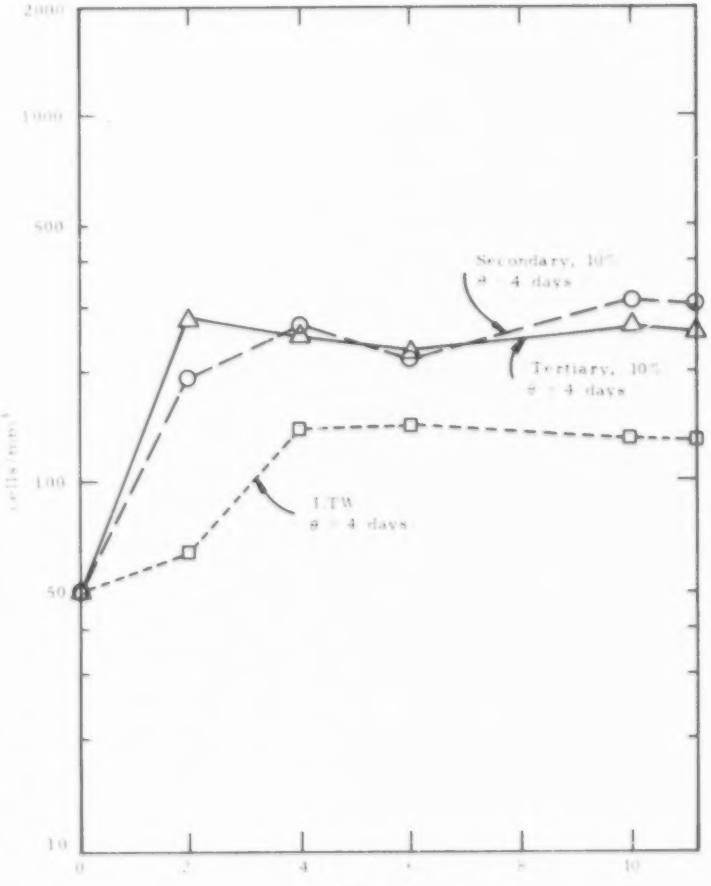


FIGURE 5-6 CELL CONCENTRATION WITH TIME FOR 10% STPUD EFFLUENTS IN CHEMOSTAT CULTURES

The critical dilution rate at which washout occurs was not revealed by the limited range of dilution rates on which Figure 5-7 is based. However, it appears that steady state of cells is difficult to maintain at dilution rates of less than about 0.1 day^{-1} and greater than about 0.35 day^{-1}.

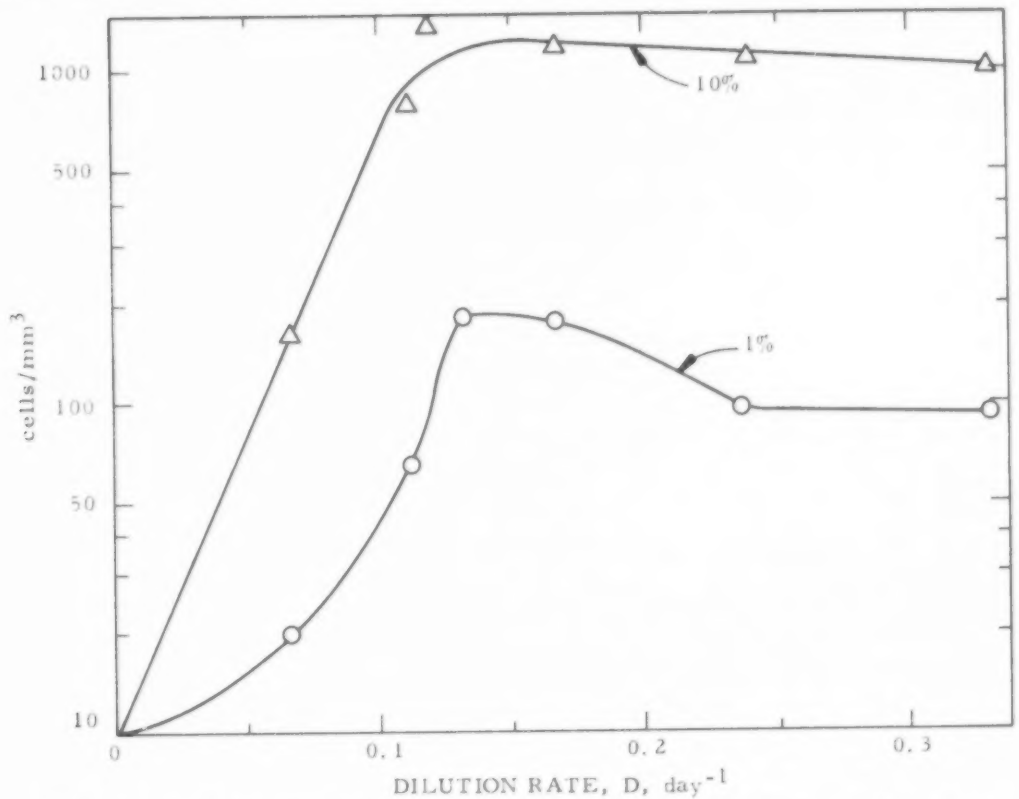


FIGURE 5-7 CELL CONCENTRATION AS A FUNCTION OF DILUTION RATE AT 1% AND 10% CONCENTRATION OF SECONDARY EFFLUENT

KINETIC CONSTANTS

The growth rate in response to different sources and concentrations of nutrients in continuous culture (chemostat) assays was evaluated in terms of the kinetic characteristics of the test organism S. gracile. The kinetic constants of concern include the maximum growth rate, $\hat{\mu}$, (day^{-1}); the saturation constant (Michaelis-Menten constant), K_s, ($\mu g/\ell$); the yield coefficient, Y, (grams of cells produced/gram of substrate removed); and the decay constant, k_d, (day^{-1}).

Evaluation of $\hat{\mu}$ and K_s

Figure 5-8 shows the relation between reciprocal specific growth rate and reciprocal nutrient concentration for sewage effluents when nitrogen was assumed to be, i.e., N/P ≤ 10. The values of μ were computed from the observed values of dilution rates and an assumed value of k_d of 0.05 day^{-1} by Equation 4, Chapter II. From the intercept and slope of the regression curve the maximum growth rate and saturation constant for S. gracile in sewage were then found to be:

$$\hat{\mu} = 0.267 \text{ day}^{-1}$$

$$K_s = 59 \ \mu g\text{-N}/\ell$$

at a significance level of 0.05.

Figure 5-9 shows the same reciprocal relationship as Figure 5-8, when the nutrient is chemical nitrogen added to LTW in the same concentrations as found in the sewages assayed. Values of μ were computed from observed dilution rates and the assumed value of k_d as before. In this case the Michaelis-Menten rate constant and coefficient were:

$$\hat{\mu} = 0.265 \text{ day}^{-1}$$

$$K_s = 14 \text{ } \mu g\text{-}N/\ell$$

at a significance level of 0.1.

It is of interest to note that for $\hat{\mu}$ there was no significant difference between sewage and chemical nutrients (N and P) at the same concentration as in sewage (0.267 vs 0.265 day^{-1}); however, the K_s value for chemical nutrients (14 μg-N/ℓ) is less than one-third the K_s value for sewage (59 μg).

An attempt to evaluate the growth constants for phosphorus limiting conditions (N/P = 15) by the addition of chemical phosphorus to LTW was not successful. The data points were widely scattered and it appeared therefore that phosphorus was not growth limiting.

Evaluation of Y and k_d

Data presented in Table 5-2 are plotted in Figures 5-10 and 5-11 for the purpose of determining the yield coefficient, Y, and the decay constant or coefficient, k_d, for the test alga *S. gracilis* in sewage effluents.

Figure 5-10 shows the relation between dilution rate and nitrogen removal. By least squares regression analysis a value of yield coefficient of 6.67 μg cells produced/μg of nitrogen removed was obtained. This value corresponds roughly to a nitrogen content of 15% in the algal cells. As noted in Table 4-3 (Chapter IV) the nitrogen content of algal cells is reported to range between about 1 and 10% (dry weight). Moreover, it is generally conceded that phosphorus cannot be limiting when the N/P ratio is less than 7. In sewage effluents this ratio is more nearly 2. Therefore, the observed nitrogen content of 15% appears to be high, which would mean that the calculated value of 6.67 μg cells/μg of nitrogen removed is too low. The correlation coefficient reported in Figure 5-10 is 0.492. This tends to confirm the hypothesis that Y may be variable rather than constant.

Decay coefficients, k_d, estimated for sewage effluents are also shown in Figures 5-10 and 5-11. In both nitrogen and phosphorus the values of k_d are calculated to be negative (- 0.125 day^{-1} for N, and - 0.084 day^{-1} for P).

Occasionally negative values of k_d have been reported by other investigators attempting to determine decay coefficients for bacterial systems [20]. Such values, however, are not consistent with the concept of materials balance, which simply states that the net growth rate (mass) is equal to the specific growth rate (mass) minus the rate of loss of mass by decay. Thus the values of k_d determined as in Figures 5-10 and 5-11 should be positive rather than negative.

The explanation of this failure of experimental data to support or verify rational concepts lies perhaps in the way algal cells behave under continuous illumination. Tamiya [21] has shown that *Chlorella* cells have low efficiency to utilize light energy when continuously illuminated and that nutrient utilization is related to energy utilization. Maddux [22] bears out this observation and concludes that growth rates are a function of a balance of light, temperature, and nutrition. Results reported by Chu [23] suggest that nitrogen concentration affects the amount of chlorophyll formed, hence the ability of the organism to utilize light and nutrients might be reduced.

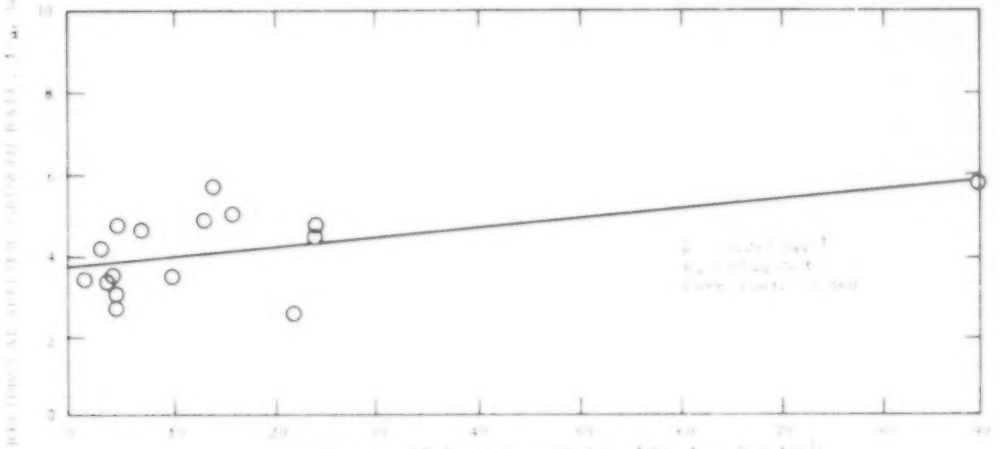


FIGURE 5-8 RECIPROCAL PLOT $1/\mu$ vs $1/S_1$ FOR ASSUMED NITROGEN LIMITING CONDITION OF WASTE WATER EFFLUENTS

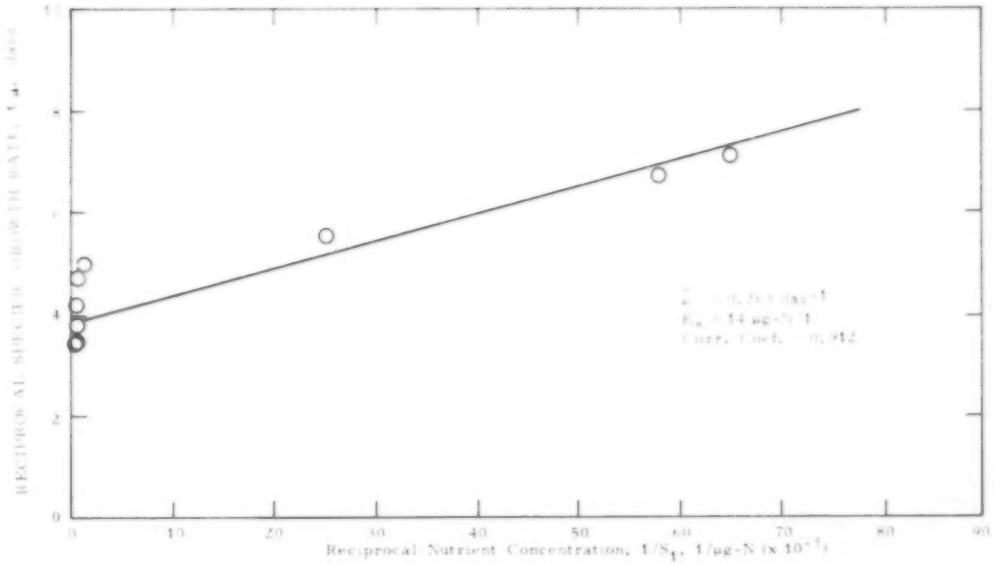


FIGURE 5-9 RECIPROCAL PLOT $1/\mu$ vs $1/S_1$ FOR ASSUMED NITROGEN LIMITING CONDITION OF CHEMICAL NUTRIENTS

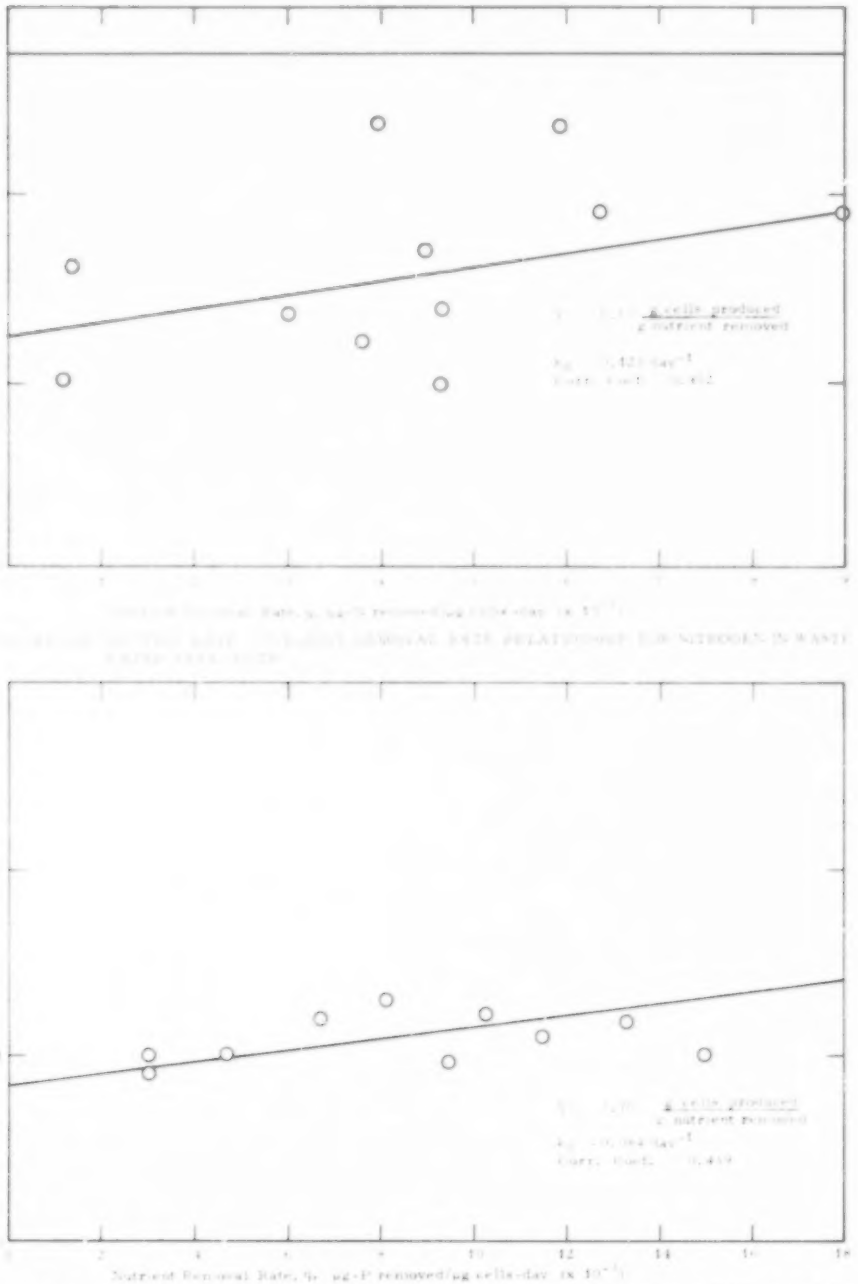


FIGURE 5-11 DILUTION RATE - NUTRIENT REMOVAL RATE RELATIONSHIP FOR PHOSPHORUS IN WASTE WATER EFFLUENTS

The foregoing observations indicate that the cell mass produced by any given amount of nutrient is not a constant but varies with its physiological state and ability of the organism to utilize light energy. Under continuous lighting the mass of any specific number of cells is smaller than under normal outdoor day and night cycles. Hence, if, as in the assays herein reported, cell mass is determined by multiplying cell count by some apparently reasonable conversion factor, the calculated cell mass may be too great. The effect of this error in the equation $1/\theta = Yq - K_d$ (Equation 6, Chapter II) is to make q too small. For a given value of $1/\theta$, then, the effect of a small value of q is to decrease the value of K_d necessary to make the equation balance. Such a decrease may well make K_d negative. In fact, some observers state that K_d will always be negative when algal cells are grown in chemostats under continuous illumination.

This explanation is in accord with the calculated yield coefficient shown in Figure 4-10, which indicates that the value of this coefficient was too small.

The fluctuation leading to the low values of correlation coefficient reported in Figures 5-10 and 5-11 may be attributed to two factors: 1) the normal fluctuation within an algal population in relation to nutrient concentration, and 2) the limitations in the accuracy of cell counting and chemical analysis of nutrient samples when nutrient concentrations are low and the resulting cell concentration in the chemostat is likewise low. Growth of organisms on the chemostat walls may also produce errors. Finally, the assumption that ITW has a constant nutrient concentration is shown later by the experiments to be invalid.

As indicated in Equation 6 (Chapter II), the value of K_d is essential to an accurate evaluation of the specific growth rate by a materials balance approach. In the presence of the assumption that Y is a constant, and in the absence of sufficient perfection of the chemostat test to show that a positive value of decay rate can be replicated with the test algae used, some resolution other than empirical data must be applied to the problem of the negative K_d. The alternatives are either to use the negative value computed, consider the decay rate as unimportant in the calculations, or estimate a value of K_d on some rational basis. To adopt the first of these alternatives would be to compound an error by ignoring theory and rationale; the second might be justifiable on the basis of its preference over the first; the third, however, would seem to minimize the error introduced through ignorance of an appropriate positive value of K_d. This does not mean, however, that the error is negligible. It might in reality be significantly large. For example, the difference between K_d = 0 and K_d = 0.05 day^{-1} introduced in Equation 6 would lead to a 50% difference in the value of Y for a residence time of 10 days.

For the computations of growth rate hereinafter introduced, a value of K_d = 0.05 day^{-1} is used. It is based on the normal assumption for algal systems that the decay rate of organisms might be expected to amount to 1% to 10% of the growth rate, and an observed specific growth rate of 0.5 day^{-1}.

From data presented in Table 5-5, Figure 5-12 has been prepared to show the change in dilution rate in relation to nutrient removal rate, q, for chemical nutrients when inorganic nitrogen was assumed to be limiting. From the figure the values of Y and K_d were found to be 5.4 µg of cells produced/µg of nitrogen removed, and - 0.09 day^{-1}, respectively, assuming a cell density of 54 µµg/cell (dry weight) (Chapter III). This yield coefficient corresponds to a cellular nitrogen content of about 18%.

Figure 5-13 shows the relationship between the dilution rate and nutrient removal rate, q, for phosphorus in the form of inorganic chemical. A cell weight of 54 µµg/cell would result in the value of Y being about 30 µg of cells/µg-P removed. This value of Y corresponds to a cellular phosphorus concentration of 3.3% (dry weight), which is about twice the largest value noted in Table 4-3 (Chapter IV). As with sewage effluents, a negative value of K_d appeared, the value being - 0.111 day^{-1}.

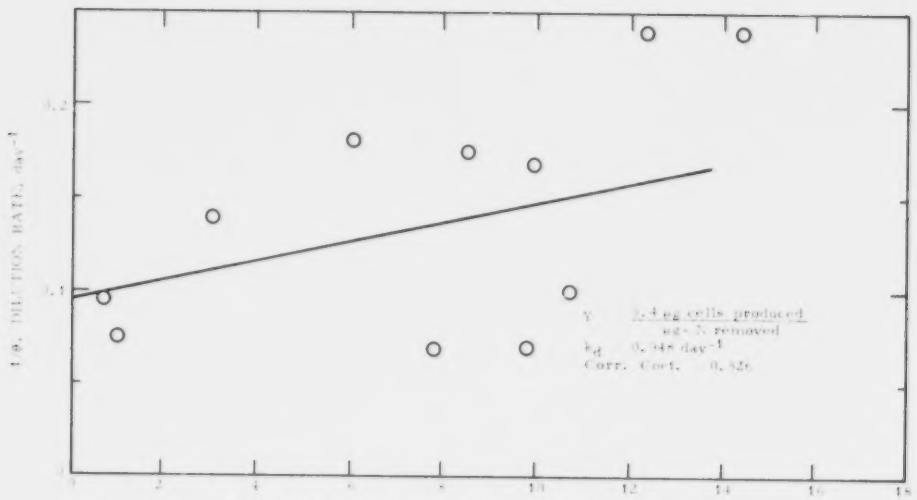


FIGURE 5-12 DILUTION RATE - NUTRIENT REMOVAL RATE RELATIONSHIP FOR NITROGEN IN CHEMICAL NUTRIENTS

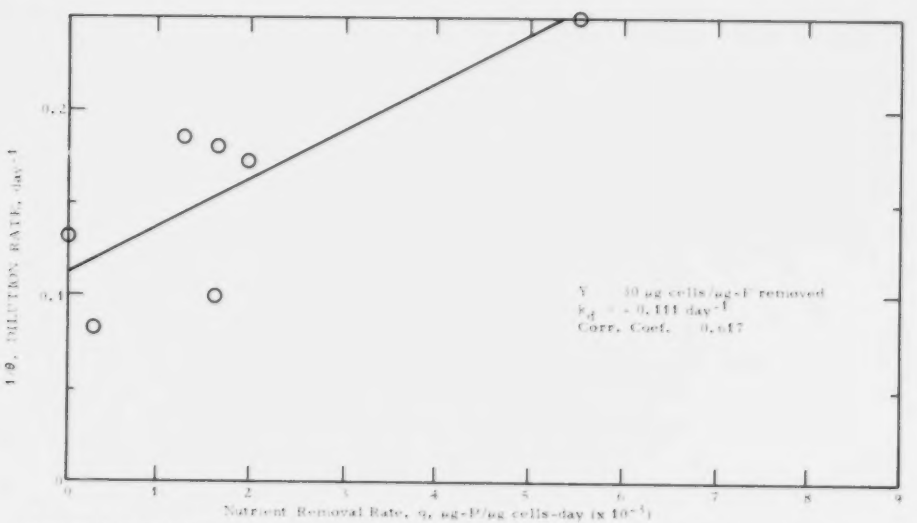


FIGURE 5-13 DILUTION RATE - NUTRIENT REMOVAL RATE RELATIONSHIP FOR PHOSPHORUS IN CHEMICAL NUTRIENTS

EVALUATION OF KINETIC CONSTANTS

The best estimates of kinetic constants and coefficients developed from the preceding data analysis for S. gracile in sewage effluents or additions of inorganic chemicals (N and P) under nutrient limiting conditions are summarized in Table 5-3.

TABLE 5-3

SUMMARY OF KINETIC GROWTH CHARACTERISTICS FOR S. gracile FOR ASSUMED NUTRIENT LIMITING CONDITIONS

Kinetic Parameter	Sewage Effluents		Chemical Nutrients	
	N-Limiting	P-Limiting	N-Limiting	P-Limiting
$\hat{\mu}$, day^{-1}	0.267	-	0.263	-
K_s, $\mu g/\ell$	59	-	14	-
Y, $\frac{\mu g \text{ cells produced}}{\mu g \text{ nutrient removed}}$	6.67	3.70	5.4	30.0
k_d*, day^{-1}	(-0.123)	(-0.084)	(-0.095)	(-0.111)

*A value of k_d of 0.05 day^{-1} is assumed in all cases.

The kinetic constants $\hat{\mu}$ and K_s for added nutrients were compared with those of sewage effluents, when nitrogen was assumed to be rate limiting. Sewage effluents yielded a maximum specific growth rate of 0.267 day^{-1} while the value of $\hat{\mu}$ for added or synthetic nutrients was found to be 0.263 day^{-1}. These rates indicate that a period of about 2.6 days is required for S. gracile to double its biomass when nitrogen is available in adequate amounts and phosphorus is not limiting. The concentration at which nitrogen limits growth of S. gracile is indicated by the values of the saturation constant, K_s. Figure 5-9 shows this to be 59 μg-N/ℓ for sewage effluents, and Figure 5-10 shows K_s to be 14 μg-N/ℓ for added or synthetic nutrients. It is at or above these minimum concentrations that S. gracile can theoretically double its biomass in the 2.6-day period. Inasmuch as the growth rate $\hat{\mu}$ was essentially the same in the two cases, this may be evidence of some toxic factor in sewage, as postulated in Chapter IV.

From Table 5-1 it may be noted that the concentrations of inorganic nitrogen in Lake Tahoe average about 66 μg-N/ℓ. Moreover, the experiments reported in Table 5-2 and the results in Figures 5-5 and 5-6 show that S. gracile was able to maintain steady state in LTW.

The values of maximum growth rate $\hat{\mu}$ (0.267 and 0.263 day^{-1}) appear to be somewhat low. In evaluating these findings, it must be considered whether such low values result from the imperfections of experimental equipment and assumptions concerning the value of the decay coefficient (k_d) or from some characteristic of the nutrient. The assumed value of 0.05 day^{-1} might be the upper limit; however, the effect of equating k_d to zero would be to increase the value of $\hat{\mu}$ to about 0.3 day^{-1}.

Figure 5-7, based on direct observation of growth rates, shows a maximum dilution rate of 0.33 day^{-1}. This means that the maximum value of $\hat{\mu}$ for 10% and 1% Reno secondary effluent cannot exceed (0.33 + k_d) day^{-1}. Inasmuch as the fitted curve (Figure 5-7) is not an envelope of maximum observations, the computed value of $\hat{\mu}$ should be less than (0.33 + k_d) day^{-1}. On this basis, it can be concluded that the values of 0.267 and 0.263 day^{-1} are reasonable estimates. The error in evaluating k_d

raises only the question of whether the generation time of S. gracile in LTW is 2 or 2.3 days - a difference probably within the limits of accuracy of the method.

In interpreting the experimental data it was assumed that nitrogen is one of the limiting substances. Although it is shown that phosphorus was not the limiting factor, or that the amount in LTW was adequate, the possibility that some growth factor other than nitrogen was limiting was not ascertained in this study. Possible factors include lack of trace elements, low concentration of nitrogen, or some factor in the LTW itself which is inhibitory to the growth of S. gracile in a unicellular culture. The data presented do not promise an answer. However, the objective of the experiment was to determine the response of LTW to added nutrients, the conclusion that additions of low concentrations of nitrogen can stimulate growth of S. gracile at a rate sufficient to double the biomass of such organisms in some two days is significant and justifies further study with more diverse heterogeneous algal populations under actual lake conditions.

The values of yield coefficients obtained for sewage effluents and chemical nutrients were 6.67 and 5.4, respectively, expressed as g cells produced/g-N removed. The corresponding value of cellular nitrogen content was within reasonable limits and hence the yield coefficients based on nitrogen are realistic, whereas the yield coefficients for phosphorus appear to be low and are likely in error. The values of saturation constant, Ks, indicate that inorganic nitrogen added as a pure chemical supported algal growth at a lower limiting concentration than did the corresponding concentration of nitrogen present in sewage effluents.

CHAPTER VI

POND ASSAYS

INTRODUCTION

Methodology

Experimental ponds designed to simulate the shallow portions of Lake Tahoe for purposes of extrapolating the results of laboratory flask and chemostat assays to lake conditions consisted of eight fiberglass-coated wooden tanks each 20 ft long, 4 ft wide, and 4 ft deep, with a water depth of 3.5 ft and a capacity of about 7950 liters (2100 gal). The ponds were installed on a wooden platform adjacent to the south wall of the Fish and Game hatchery building, which housed the laboratory, with the long axis on the meridian. The general layout of the installation is shown schematically in Figure 6-1.

At the north end of each pond a weather-tight, ventilated cabinet was constructed to house the nutrient sample containers, feed pumps, electrical controls and outlet boxes. A Jaccuzi Whirlpool submersible pump was installed in each pond and operated continuously during assay experiments to keep the pond moderately well mixed. The volume of water in the pond was maintained constant by a 1-1/2-in. galvanized iron standpipe threaded into an upside down floor flange built into the pond bottom near the south end of the structure. This standpipe could be removed for draining and cleaning of the pond between experiments, or assays.

LTW used in the pond assays was pumped directly from the lake by means of a centrifugal pump located near the shoreline. The pump intake was placed 150 yd offshore in about 10 ft of water at 4 ft above the lake bottom. The pump discharged through 1600 ft of 2-in. PVC into a horizontal 6-in. PVC header supported on a superstructure of the cabinets at an elevation of approximately 4 ft above the pond water surface and extending across the entire 8-pond installation. Flow into each pond was by gravity through a small plastic line originating in the common header and fitted with a valve, by which the rate was controlled to provide the desired detention period, or residence time. Excess water delivered to the header was discharged over a riser spillway ring and returned to the lake via surface runoff in a natural channel draining a spring which outcropped at the site of the Fish and Game hatchery building.

At the beginning of each assay, the pond, with one exception, was washed, rinsed, and filled with LTW. The residence period was adjusted experimentally. The sample containing the enriching nutrient to be evaluated by assay was then pumped into the pond from a 5-gal plastic container by a Sigmamotor pump at a uniform rate appropriate to produce and maintain the desired concentration of sample in the LTW at the selected residence time, hence nutrient levels and growth of algae increased to steady state simultaneously. Two reference ponds containing only LTW were maintained at the same residence time as the experimental ponds.

Stock cultures of *S. gracile* for inoculation of both the experimental and reference ponds were grown in large quantity. As it turned out, however, attempts to use cell count of this test alga as a measure of the growth rate were unsuccessful. Indigenous growths, primarily diatoms, predominated in all pond assays and had to be used as indicators of growth response. An attempt was made to filter indigenous organisms from the incoming LTW by passing it through a diatomaceous earth, swimming pool filter. However, as noted in Chapter III, the pump characteristics were unsuited to the head loss involved and the season was too advanced to permit delay of experiments while a new pumping installation was provided. A comparison of seeded ponds and indigenous ponds was made, as hereinafter reported.

Chemical and physical analyses of the pond water were made at regular intervals during each experiment, or assay. Grab samples were collected just below the water surface at the midpoint of the pond. (See Appendix D.) Because of wall growth (primarily pennate diatoms) the walls and bottom of the ponds were scraped with a rubber squeegee in the late afternoon of the day prior to sampling. At 9:00 a.m. on the following morning a sample from each pond was collected, filtered through glass fiber (GF/C) for determination of suspended solids – the measure of organic growth – and for obtaining filtrate for chemical analyses. Routine chemical determinations of filtered samples included: NO_3-N, NH_3-N, Organic N, inorganic and total phosphorus. In addition, conductivity, pH, and temperature were observed.

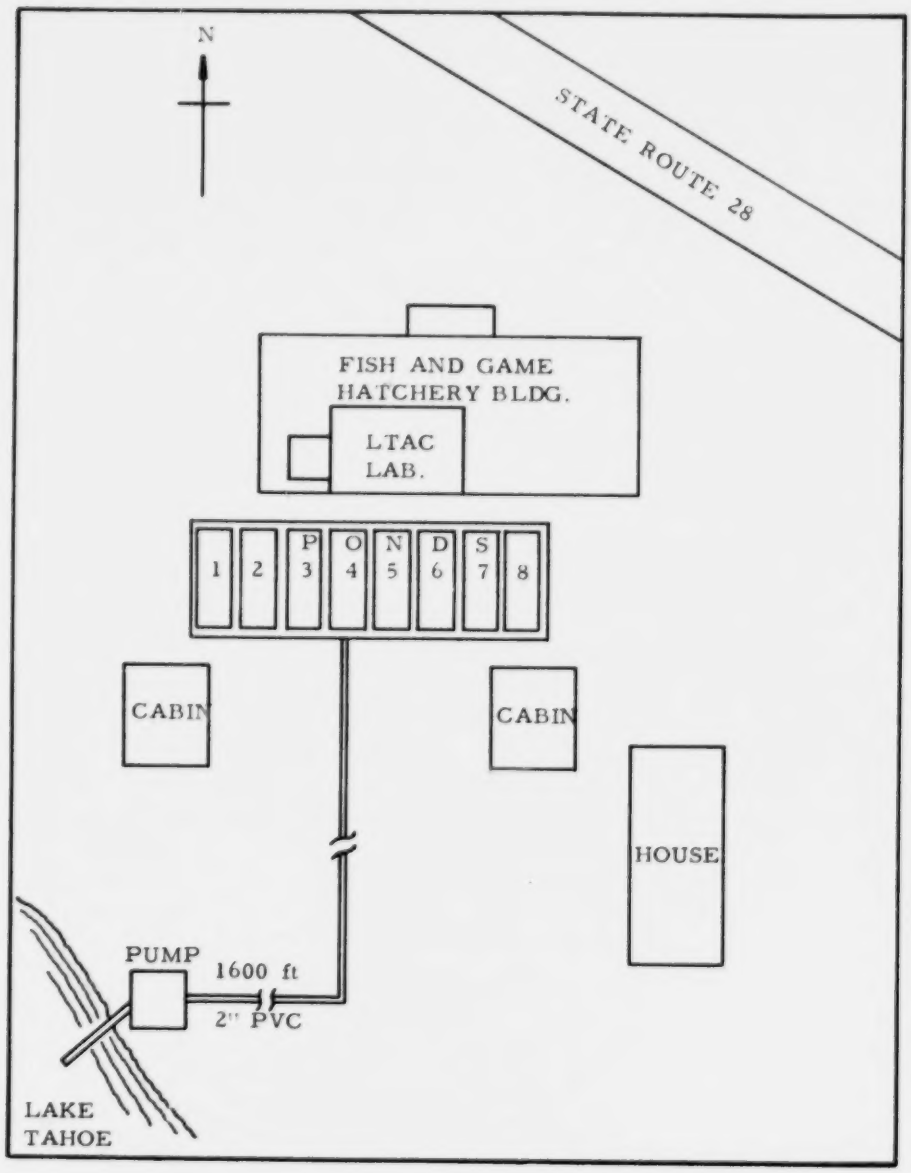


FIGURE 6-1 LAYOUT OF EXPERIMENTAL PONDS

LTW was analyzed intermittently for chemical characteristics as a control measure. Samples of sewage effluents to be introduced as added sources of nutrients in the experiments were analyzed on each collection date, as reported in Appendix E. Secondary effluent was obtained as grab samples from the clarifier effluent following activated sludge treatment at the South Tahoe Public Utilities District (STPUD) Water Reclamation Plant. Tertiary effluent from STPUD was obtained as grab samples from the effluent from the activated carbon filter units. Essentially, tertiary treatment consisted of precipitation with lime to remove phosphates, pressure filtration, and activated carbon filtration. Samples were collected as needed in approximately 50 to 75-gal lots, in 5-gal polyethylene bottles, and used within one week after collection.

Biological examination of pond samples was primarily by assessment of volatile and total suspended solids; however, several other methods were used for comparison. Conversion factors were determined for selected sets of ^{14}C uptake, aufwuchs COD*, and chlorophyll content. Phytoplankton count data were collected primarily as a cursory identification of indigenous growth observed in the ponds.

The types of analyses run in relation to the pond assays are indicated in Table 6-1, with particular reference to individual assays to which they apply.

TABLE 6-1

TYPES OF DATA AND ANALYSES OBTAINED DURING POND ASSAYS

Meteorological data, U. S. Weather Bureau[a]	
Temperature, ambient[a] and water	
Incident light[a]	
Flow rates (twice daily except on weekends)	
pH of pond water	
Conductivity of pond water	
^{14}C (twice during each assay)[b]	
Suspended solids (SS) and volatile suspended solids (VSS) (three times per week)[c]	
Aufwuchs COD (Experiments No. 5, 6, and 7 only)[b]	
COD[b], chlorophyll[b]	
Flask cultures to test biostimulatory response (Exp. 6 and 7 only)	
Phytoplankton counts and cursory identification (Exp. 6 and 7 only)[d]	
Chemical measurements of:	
NO_3^--N	BOD (input only)
NO_2^--N	COD (input only)
NH_3-N	Alkalinity (input only)
Organic N	Chloride (input only)
$PO_4^{\equiv}$-P	Reactive iron (input only)
Total P	

[a]See Appendix G

[b]See Appendix H

[c]See Appendix F

[d]See Appendix I

The experimental design used in the pond assays is summarized in Table 6-2. As indicated in the table each assay experiment made use of all eight ponds. In each experiment three ponds were used for tertiary effluent, three for secondary effluent,

*Organic material growing on the surface of glass slides suspended in the ponds.

and one or two for untreated LTW. Experiments 4 and 5 included only one reference pond because Pond 1 was used to culture S. gracile as seed for Experiments 5 and 6. Experiment 6 likewise had only one reference pond because Pond 1 was at that time used to assay secondary effluent without seeding, for comparison with seeded Ponds 7 and 8. The ponds selected to receive any particular type or concentration of effluent were changed from experiment to experiment to randomize the position effect which might exist as a result of the geometry of the installation. Ponds 2, 3, and 4 received 0.1%, 1.0%, and 1.0% secondary effluent, respectively, for Experiments 3 and 5. Ponds 6, 7, and 8 then replaced 2, 3, and 4, in Experiments 2, 4, 6, and 7. The ponds receiving tertiary effluent were the reverse order of those receiving secondary effluent.

TABLE 6-2

EXPERIMENTAL DESIGN OF POND ASSAYS

Assay No.	Residence Time (θ)	Dates	Pond No.[a]							
			1	2	3	4	5	6	7	8
2[b]	10 days	Jun 18-Jul 2	Lake Tahoe	0.1% III	1.0% III	1.0% III	Lake Tahoe	0.1% II	1.0% II	1.0% II
3	5 days	Jul 5-29	Lake Tahoe	0.1% II	1.0% II	1.0% II	Lake Tahoe	0.1% III	1.0% III	1.0% III
4	5 days	Aug 2-16	Seed Pond	0.1% III	1.0% III	1.0% III	Lake Tahoe	0.1% II	1.0% II	1.0% II
5[b]	5 days	Aug 16-30	Seed Pond	0.1% II	1.0% II	1.0% II	Lake Tahoe	0.1% III	1.0% III	1.0% III
6[b]	10 days	Sep 1-Oct 4	1.0% II	0.1% III	1.0% III	1.0% III	Lake Tahoe	0.1% II	1.0% II	1.0% II
7	5 days	Oct 4-Nov 1	Lake Tahoe	0.1% III	1.0% III	1.0% III	Lake Tahoe	0.1% II	1.0% II	1.0% II

[a] II is secondary effluent and III is tertiary effluent

[b] All ponds were seeded initially with Selenastrum gracile

As shown in Table 6-2, residence periods of 5, 5, and 10 days were used in pond assays. As noted in a preceding paragraph, the ponds were normally drained, cleaned, and refilled between experiments or assays. A single exception to this procedure occurred in the case of Experiment 7. Because of the onset of storms and freezing weather the residence time (θ) was changed from 10 days in Experiment 6 to 5 days in Experiment 7 by adjusting the flow.

Although all pond communities began to resemble indigenous communities as steady state approached (the difference being only the occasional appearance of S. gracile among the primarily pennate diatom communities in seeded tests), a comparison of seeded and indigenous ponds was made. In assays 2, 5, and 6 the ponds were seeded with S. gracile at the beginning of the test. Experiment 2 was seeded with S. gracile

grown in Bristol's medium in a bathtub indoors, continuously illuminated with fluorescent lighting. Experiment 5 was seeded with the same organism grown in Pond 1 outdoors using Skulberg's medium with trace elements supplied by adding 1 liter of secondary sewage effluent [14]. In Assay 6 ponds 2-8 were seeded with S. gracile grown in Pond 1. Inadvertently, phosphate was omitted from the substrate, which apparently limited the growth; however, nitrogen was in excess. This omission was discovered when the experiment (Assay 6) was underway. The ponds were then drained to a depth of 1 ft, refilled with LTW, and the assay continued.

Experiment 1 is not included in Table 6-2 nor in the evaluation of results which follows because the inner surfaces of the ponds were not scraped to disperse the organisms responsible for the measured loss of nutrient in the system.

The ponds were assumed to be reactors continuously receiving known amounts of nutrients and operated at a steady state where growth is balanced by the outflow of organisms. This assumption implies that only one factor limits the growth of the living material. However, there were many variables in the pond system because the ponds were operated under natural outdoor conditions. Among these variables were sunlight; temperatures; allochthonous materials*; and the composition of lake water, sewage effluents, and the pond community.

In the ponds, as in any continuous culture, there were two independent variables; input concentration, S_0, and residence time, θ, which together regulated the concentration of nutrients, S_1, and biomass, X_1, in the ponds. As discussed in Chapter II, the biomass and nutrient relationships may be described by the microbiological growth constants and coefficients $\hat{a}$, K_s, Y, and K_d, which vary with algal species and type of nutrient.

In the pond assays, values of the different variables were obtained as follows: The residence time, θ, or mean cell age, θ_c, was fixed by the flow rate, F, and volume, V, of the pond, i.e., V/F. The biomass, X_1, was estimated as VSS. The input, S_0, and pond, S_1, concentrations of NO_3-N, NH_3-N, NO_3 plus NH_3 as N, total N, PO_4-P, and total P were determined and used for calculating nutrient relations. The growth constants were determined for the different kinds of nutrients from the least squares fits for the steady state relations as derived in Chapter II.

RESULTS

Environmental Parameters

Data on the environmental variation (Appendix C) occurring during the pond assays are shown graphically in Figure 6-2. From this plotting it is evident that the temperature of the pond water followed the same general trend as the median ambient air temperature. The air temperature, however, shows the greater variation, as might be expected from a consideration of the thermal capacity of water. The total amount of light incident on Lake Tahoe [24] is also shown in Figure 6-2. In general, the pattern is similar to the ambient air temperature data.

SS measurements for ponds containing untreated LTW (reference ponds) together with precipitation are shown in Figure 6-3. Storms invariably resulted in large increases of suspended material. Some of this was carried in with rain water, but much of it was blown into the ponds by winds associated with rain storms. The nonvolatile particulate material entering the pond was appreciable, as can be seen by comparing the amount of SS with VSS in the reference ponds (see Figure 6-4). However, it was observed that considerable wind-carried, organic particulate material such as pollen, insects and pine needles was entering the ponds independently of storms, causing some variation in VSS.

*Materials of foreign origin.

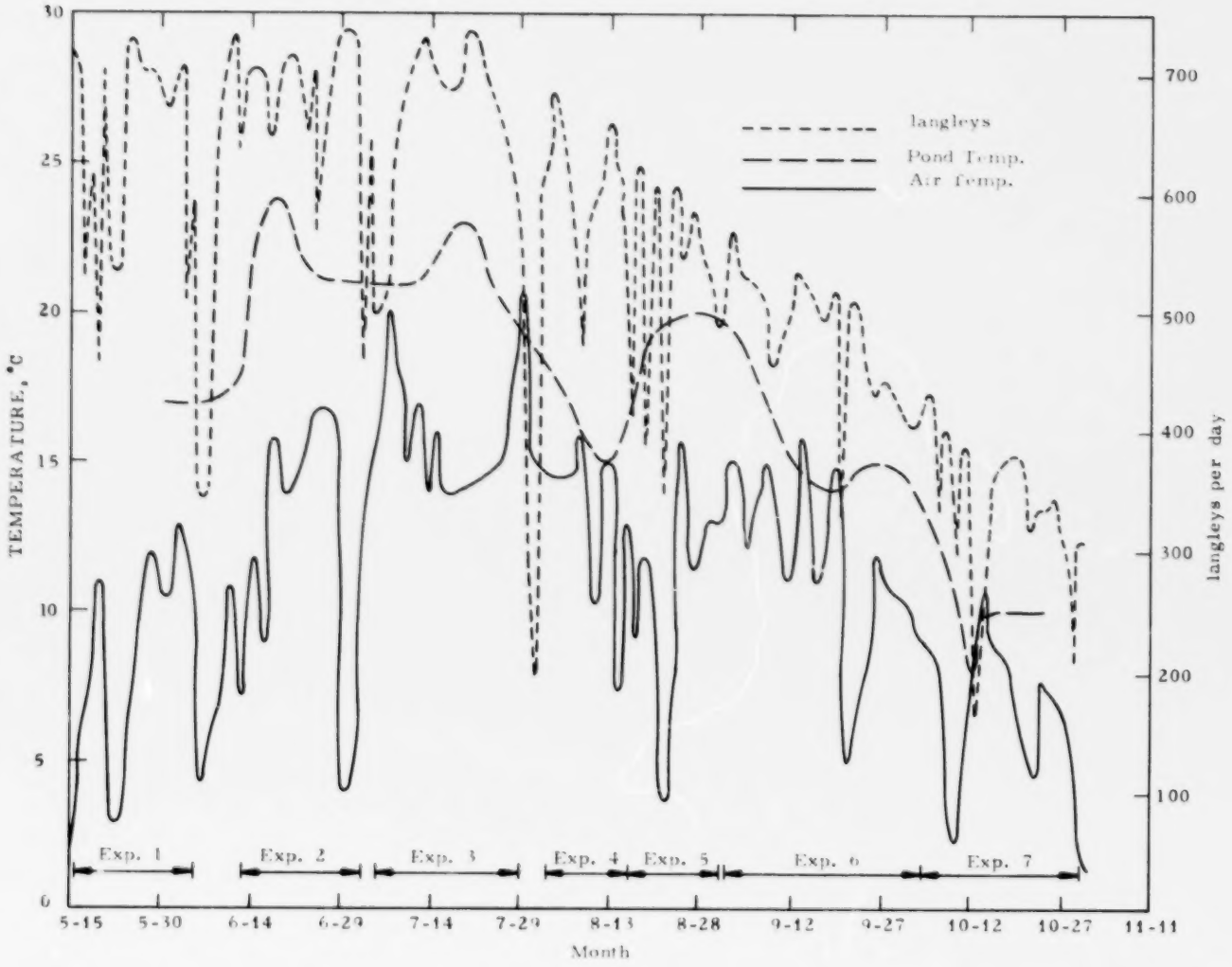


FIGURE 6-2 VARIATION IN ENVIRONMENTAL FACTORS DURING POND ASSAYS

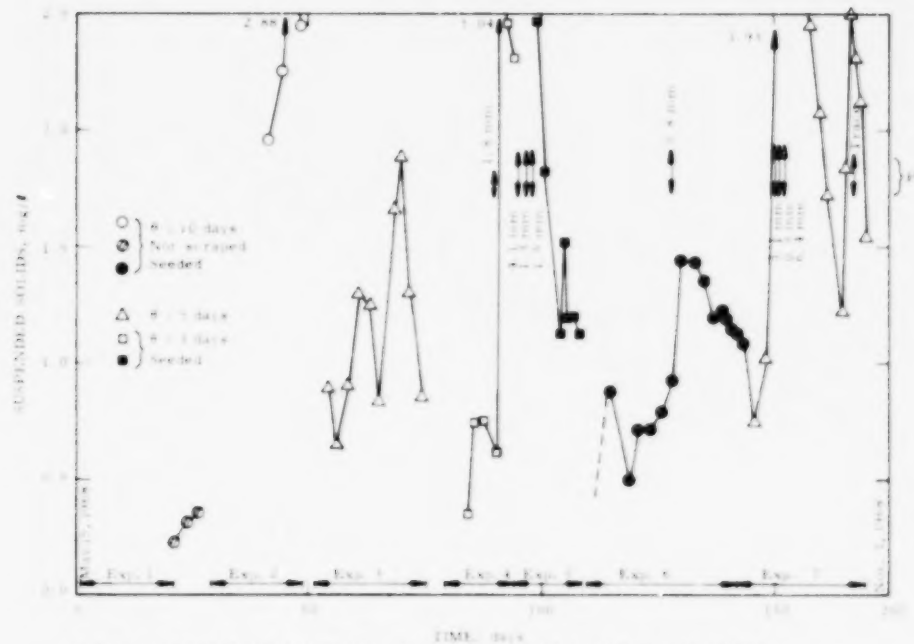


FIGURE 6-3 SUSPENDED MATERIALS IN PONDS RECEIVING LAKE TAHOE WATER

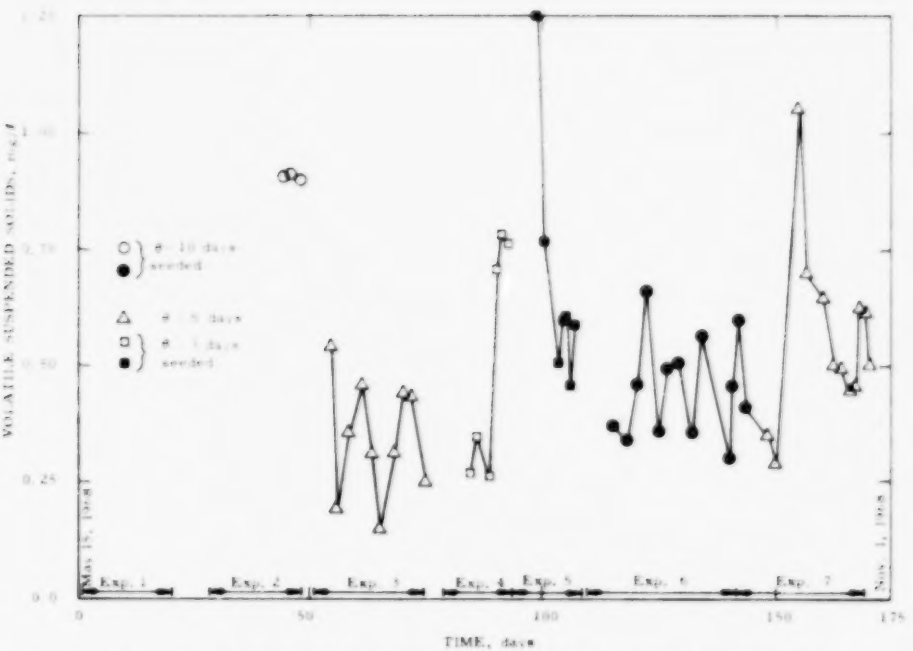


FIGURE 6-4 VOLATILE SUSPENDED MATERIALS IN PONDS RECEIVING LAKE TAHOE WATER

Variations in input waters did not have an appreciable effect on the concentrations of nutrients in the ponds because sudden changes in concentration do not occur in continuous flow systems and any variations tended to be smoothed out over a period of time. In those ponds that were seeded, an initial input of nutrient carried over with the seed had to be included in the total nutrient ascribed to the sewage in calculating input concentrations, S_o; hence the variations are included although not identified.

Biomass Estimation

To give a more complete estimation of biomass, several different techniques were used concurrently. The most important of these are listed in Table 6-3. Equations relating the different techniques were calculated using least squares with y the assumed dependent variable and x the assumed independent variable. For measurements of SS, VSS and COD, enough replications of samples were obtained to determine the reproducibility of those biomass measurements (Table 6-4). The measurement of VSS was used in this study to estimate biomass because it was simple and could be done without a loss of precision.

TABLE 6-3

LINEAR RELATIONS BETWEEN DIFFERENT ESTIMATES OF BIOMASS

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Corr. Coef.	Equation[a] y = mx + b
Chemical Oxygen Demand, mg/ℓ [COD]	Suspended Solids, mg/ℓ [SS]	27	0.95	COD = (0.78)* SS + (-0.55)
Chemical Oxygen Demand, mg/ℓ [COD]	Volatile Suspended Solids, mg/ℓ [VSS]	27	0.95	COD = (1.43)* VSS + (-0.39)
mgC/ℓ-day [^{14}C]	Volatile Suspended Solids, mg/ℓ [VSS]	91	0.54	^{14}C = (49.6)* VSS + (-7.42)
Volatile Suspended Solids, mg/ℓ [VSS]	Diatoms, Number/mm^3 [D]	128	0.83	VSS = (0.14)* D + (0.85)*
mgC/ℓ-day [^{14}C]	Chlorophyll, μg/ℓ [C]	23	0.95	^{14}C = (20.7)* C + (-29.6)
mgC/ℓ-day [^{14}C]	Diatoms, N Number/mm^3 [D]	32	0.83	^{14}C = (7.14)* D + (2.20)
Chlorophyll, μg/ℓ [C]	Volatile Suspended Solids, mg/ℓ [VSS]	23	0.84	C = (3.58)* VSS + (0.24)
Chlorophyll, μg/ℓ [C]	Diatoms, Number/mm^3 [D]	16	0.85	C = (0.39)* D + (1.90)
Autwuchs COD, mg/cm^2 [P]	Diatoms, Number/mm^3 [D]	10	0.85	P = (0.0205)* D + (0.016)
Aufwuchs COD, mg/cm^2 [P]	Volatile Suspended Solids, mg/ℓ [VSS]	13	0.92	P = (0.037)** VSS + (0.026)

[a]If marked with (*), the value of constant is significantly different from zero at P > 0.99.

If marked with (**), the value of constant is significantly different from zero at P > 0.95. All other values of constants considered equivalent to zero since P < 0.8 and in most cases P < 0.6.

The relations between the different estimates of biomass indicate that all measures give relatively good correlation except VSS with productivity ($\mu gC/\ell$-day, ^{14}C) and with Aufwuchs COD. Productivity is a rate and is therefore not strictly comparable with the other measurements which refer to "standing crop". Since chlorophyll correlates well with ^{14}C and relatively well with diatoms, it is probable that it represents a measure of the physiological state of the cells, i.e., cells which are faster growing (having higher productivity) would have a higher chlorophyll content, other conditions being equal. That the diatoms represent only a fraction of the total biomass is evident from the fact that the equation relating VSS and diatoms has a positive intercept which is significantly different from zero. All the other intercepts are not significantly different from zero.

TABLE [illegible]

PRECISION OF BIOMASS MEASUREMENTS
[No. of replicates indicated by ( )]

Date	Aufwuchs No.	Form	Suspended Solids		Volatile Suspended Solids,		COD	
			Mean (mg/ℓ)	C_V (%)	Mean (mg/ℓ)	C_V (%)	Mean (mg/ℓ)	C_V (%)
August 2[illegible]	[illegible]	[illegible]	[illegible] (6)	10.[illegible]	2.56 (3)	5.5	-	-
August 30	[illegible]	[illegible]	1.[illegible] (7)	1.9	-	-	1.7[illegible] (5)	[illegible]
September 2[illegible]	[illegible]	[illegible]	[illegible] (5)	2.1	-	-	0.22 (5)	12.[illegible]
September 2[illegible]	[illegible]	7	[illegible].55 (5)	5.5	-	-	3.07 (4)	4.6
November 1	7	5	1.78 (6)	2.2	0.49 (5)	5.7	-	-
November 1	7	8	11.[illegible]8 (6)	[illegible].2	[illegible].09 (5)	6.5	-	-
Weighted mean C_V (all replicates considered)				(35)	4.[illegible]7	(13)	6.04	(12)	7.00

The Aufwuchs COD analysis is relatively crude but gives a fairly good correlation with diatoms. Since the other parameters correlate reasonably with the diatom counts, one can conclude that the algal community which developed in the ponds was composed primarily of diatoms.

Pond Comparison. At steady state the biomass levels in duplicate ponds should theoretically be the same. An analysis of variance for paired ponds for the different assays is shown in Table 6-5. For purposes of identification the letters W (west) and E (east) are used to denote the relative positions of the paired ponds.

TABLE 6-5

COMPARISON OF BIOMASS ESTIMATES BETWEEN
PONDS RECEIVING THE SAME INFLUENTS

Assay Number	Mean Biomass Value[a], mg/ℓ VSS						
	LTW		1% II		1% III		1% II
	W	E	W	E	W	E	Pond 1
2	0.88	0.93	2.77[b]	2.17[b]	1.77	1.59	-
3	0.39	0.33	3.09	2.23	1.72	1.24	-
4	-	-	2.32	2.09	0.76	0.89	-
5	-	-	3.00	2.45	1.47	1.34	-
6	-	-	2.65	2.36	1.04	0.92	2.20
7	0.58	0.48	4.10	5.10	0.76	0.68	-

[a]II = secondary effluent; III = tertiary effluent; LTW = Lake Tahoe water.

[b]Mean values significantly different from each other at $P > 0.95$.

Statistically, no significant differences were observed except in Assay No. 2 of 1% secondary effluent.

LTW Pond Reactors. Mean values of biomass in terms of VSS observed at steady state in pond assays of LTW are summarized in Table 6-6 in order of ascending magnitude. As indicated by the brackets in the table only the smallest observation (0.36) and the largest (0.91) are significantly different than others observed. Thus the extremes of the range of VSS values occurred at a time when water temperature were the highest and in the months (June and July) when the length of daylight and the total illumination are the greatest. Consequently it is evident that light and temperature were not limiting to growth of the organisms (mostly pennate diatoms) comprising the biomass.

Seasonal variation in the nutrient content of LTW such as reported in Chapter IV was not evident in the limited number of observations made in June and July (see Figure 4-2). Therefore nutrient variation in the lake water can not be identified as responsible for the difference in biomass observed. However, the effects of pollens and other airborne organic matter reaching the ponds, or of variability of the organic content of the influent LTW, cannot be evaluated. Hence these sources cannot be ruled out as possible causes of the significant differences reported in Table 6-6.

TABLE 6-6

MEAN BIOMASS AS VSS IN POND ASSAYS OF LAKE TAHOE WATER
(Brackets indicate values not significantly different at 95% level)

Assay Number	3	6	7	5	4	2
Month	July	Sept	Oct	Aug	Aug	June
Residence Time (θ) (days)	5	10	5	3	3	10
Mean Water Temp. (°C)	23	15	11	17	17	21
Mean Light Intensity (Langleys/day)	658	480	334	526	601	654
Mean VSS (mg/ℓ)	0.36	0.44	0.53	0.55	0.63	0.91

There is no way to determine what might have been the combined effect of temperature, light, residence time, and transient nutrient concentration in the input LTW. These factors along with the natural variability of living organisms and the experimental error inherent in sampling a system in which the indicator organisms are normally attached to the pond walls rather than in the circulating water mass, may be responsible for the variation in VSS. Viewed in the light of possible sources of variation, the range of values of VSS observed, with the exception of Assay No. 2, may not be excessively great.

Nonsteady State Analysis

Observation of data began in pond assays immediately upon star ing the experiment. Therefore information was obtained when the system was in a transient state as well as in steady state. By relating changes in biomass or nutrient concentration to time, and by reasonable assumptions in materials balance equations, these transient data may be analyzed to obtain information on cellular growth and nutrient utilization in the ponds under transient conditions. Two pond assays (Nos. 5 and 6) were especially suited to such analysis because of unique conditions which occurred at the beginning of each. The first (No. 5) was a seeded assay at a residence time of 3 days and reveals a variation in biomass, or suspended material, with time. The second (No. 6) was also a seeded assay, but with a large carry over of NO_3^- nitrogen as a result of the seeding. Hence it provided information on nutrient utilization with time.

Assay No. 5 – Biomass Correction Procedure. As previously noted in Table 6-1, Assay No. 5 involved 6 ponds containing various concentrations of secondary and tertiary sewage effluents, plus one pond of LTW. If it is assumed that allochthonous material, whether rain-carried or windblown, enters all ponds in equal amounts, and that the amount of nutrients contributed by LTW is the same in all ponds, it is possible to subtract out the extraneous and baseline contributions of SS and nutrients to each pond and so evaluate the change in biomass during both transient and steady state conditions. The results of such an approach are reported in Figure 6-5.

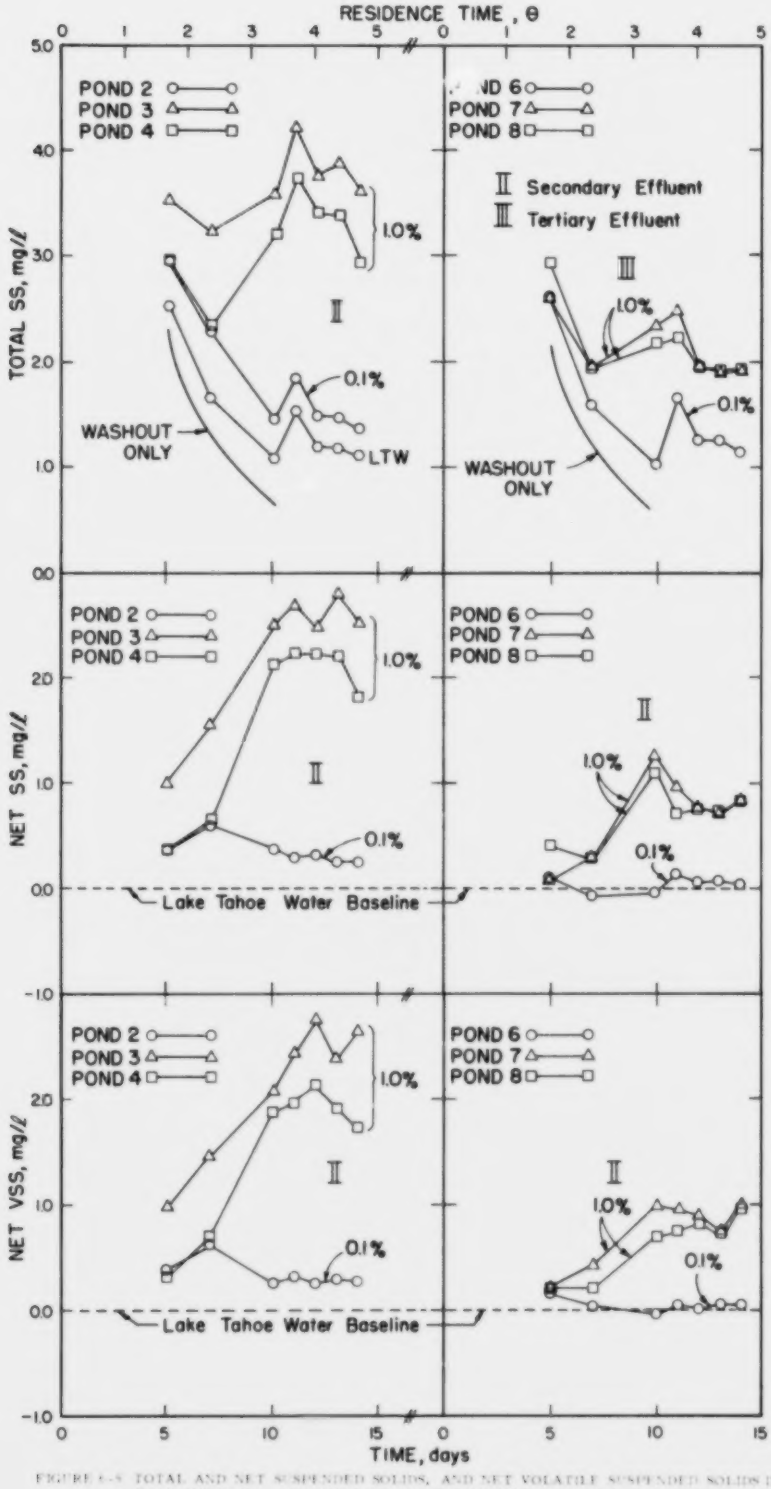


FIGURE 6-5 TOTAL AND NET SUSPENDED SOLIDS, AND NET VOLATILE SUSPENDED SOLIDS IN POND ASSAY NO. 3 OF VARIOUS CONCENTRATIONS OF WASTE WATER EFFLUENTS

The upper section of Figure 6-5 presents the change in total SS with time in all ponds without regard to the source or nature of the solids. It shows that there was a progressive loss of the initial SS until a steady state appeared to have been reached. This reflects the washout of an initial inoculum of S. gracile cells in each pond until the algal community had undergone a change in number and composition to a level supportable by the nutrients present in the ponds. For the ponds containing 0.1% concentration of waste water effluents the initial decrease in SS paralleled the theoretical washout of solids until about 5-10 days had passed at which time the growth equaled the washout. By that time it was observed that diatoms and other species in lesser numbers had largely replaced the Selenastrum; probably because Selenastrum was reproducing at a very low rate and it took some time for the indigenous species to reach a steady state.

Subtraction of the values of SS observed in the LTW reference pond from those observed in the test ponds resulted in the values of SS plotted in the middle section of Figure 6-5. Here the curves show the growth of biomass due to the excess of nutrients added with the waste water effluent. Relatively good agreement is evident between replicate ponds and the pattern is similar to that which might be expected; i.e., an increase (growth > washout) during a transient state which leads to a steady state (growth = washout). That this concept of "net SS" may be a valid one can be seen by comparing the middle and bottom sections of Figure 6-5. Net SS and VSS are presented in Table 6-7 and are in good agreement with each other. This shows more clearly that the subtraction did account for extraneous SS and the biomass induced by Lake Tahoe nutrients; hence the middle and bottom sections of Figure 6-5 represent the change in biomass due to the nutrients present in waste water effluents. The method seems to be a crude but reasonably accurate way of correcting for the weight of suspended material coming into the ponds as windblown or rain deposited debris.

TABLE 6-7

MEAN VALUES FOR SS AND VSS CORRECTED FOR LAKE TAHOE WATER NUTRIENTS AND ALLOCHTHONOUS MATERIALS FOR ASSAY NO. 5, θ = 3 DAYS

Pond No.	Effluent Concentration	Net SS mg/ℓ	Net VSS mg/ℓ
2	0.1% Secondary	0.31	0.27
3	1.0% Secondary	2.62[a]	2.46[a]
4	1.0% Secondary	2.12[a]	1.91[a]
6	0.1% Tertiary	0.05	0.02
7	1.0% Tertiary	0.90	0.91
8	1.0% Tertiary	0.81	0.78

[a]Ponds 3 and 4 are significantly different at $P \geq 0.95$.

Assay No. 5 also revealed a difference between ponds 3 and 4. In Table 6-5 ponds 3 and 4 appear as W and E ponds, respectively, in the column headed 1% II. There it was shown that they were not significantly different from each other. However, after subtracting the SS and VSS values observed in the LTW reference pond, a difference between the solids appears in Table 6-7. Visual observation likewise indicated that some difference existed. The reasons for the difference cannot be explained at this time.

Biomass – Influent Nutrient Relationship. An estimate of the way the amount of biomass produced varies with nutrient concentration can be obtained by plotting the net VSS shown in Table 6-7 against the corresponding values of nutrient concentration in the pond influent as shown in Figure 6-6. It includes the data for Assay No. 4 as well as those for Assay No. 5 because both involved a residence period of 3 days and the number of data points for any individual assay is necessarily small.

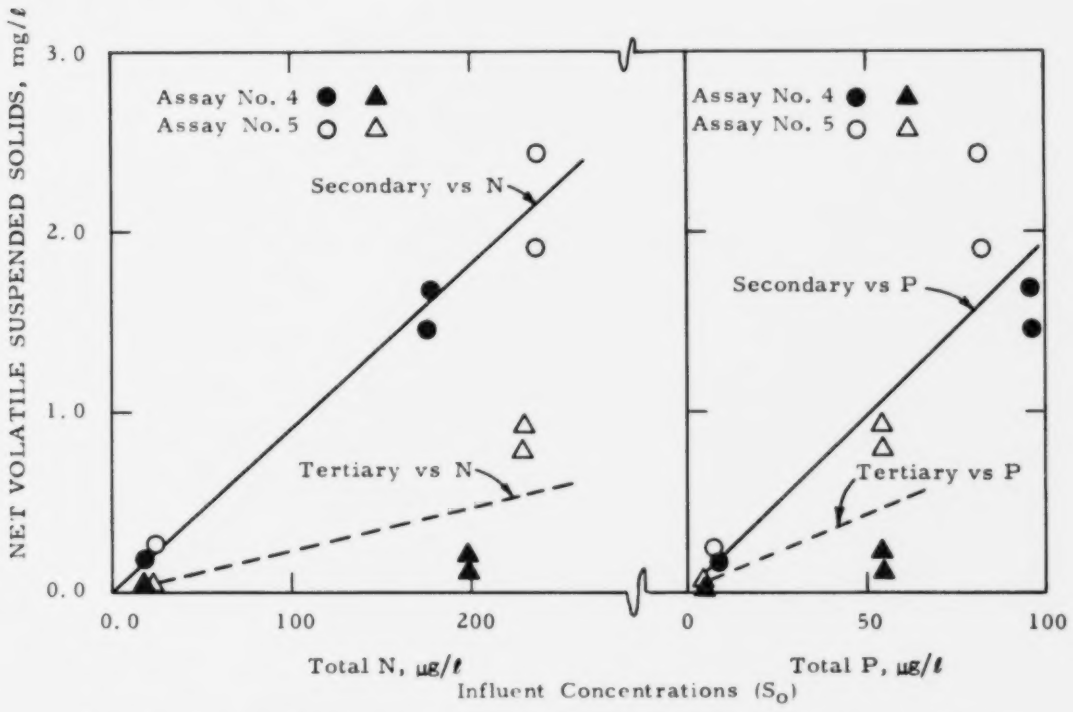


FIGURE 6-6 EFFECT OF N AND P CONCENTRATION ON THE BIOMASS. ASSAYS NO. 4 AND 5, θ = 3 days, AUGUST 1968

The curves shown in Figure 6-6 are based on the relationship between biomass and nutrient concentration:

$$X = Y (S_o - S_1) \quad , \tag{6-1}$$

which at steady state can be approximated by the relation:

$$X = Y (S_o) \tag{6-2}$$

if S_1 is close to zero.

When no nutrient is added (i.e., S_o = nutrient concentration in LTW) no additional growth would be expected in the ponds, hence the curves were arbitrarily forced through the origin in Figure 6-6.

Inspection of Figure 6-6 reveals that there is considerable scatter in the data relating the planktonic concentration to the influent nitrogen and phosphorus concentration at a residence time of 3 days. However, for secondary effluents and the two nutrients a considerably higher correlation is obtained between the net VSS and the influent total nitrogen concentration than with the total phosphorus concentration (correlation coefficient r = 0.99 for nitrogen and 0.86 for phosphorus). These data indicate that of the two, nitrogen is likely limiting which is what one would expect with a nitrogen to phosphorus ratio of about 2.5:1 in secondary effluents.

The correlation between net VSS and influent total nitrogen and total phosphorus is relatively poor for tertiary treated effluents (correlation coefficients, r, of 0.72 and 0.66 respectively). However, there is markedly less growth observed in tertiary effluents than in secondary effluents for the same concentration of nitrogen and phosphorus. This indicates that some substance other than nitrogen or phosphorus is growth limiting. Inasmuch as lime precipitation is used for phosphate precipitation in the tertiary treatment, followed by carbon filtration it is possible that other growth limiting substances such as iron, trace elements or organic growth factors may be removed by the treatment. It is also possible that some substances toxic to algal growth may have been added during tertiary treatment.

Unfortunately, reliable data on the nutrient removal ($S_o - S_1$) in the ponds was not obtained so that an accurate estimate of the yield coefficient, Y, can be made. However, using the approximate equation (6-2) the yield coefficient for nitrogen was found to be about 9 mg of net VSS (cells) produced per mg of nitrogen removed. This corresponds to a cellular nitrogen level of about 11 percent, which is a reasonable value compared to the reported nitrogen percentages shown in Table 4-1.

Assay No. 6 - Transient Nitrogen Conditions. The data from Assay No. 5 indicated that nitrogen might have been growth limiting in the ponds receiving secondary effluent. However, with tertiary treated effluent there was no clear indication that nitrogen, phosphorus or any other growth factor was limiting.

In Assay No. 6 the addition of a seed population of Selenastrum and associated unutilized nutrients in the seed culture medium produced a much higher concentration of nitrogen (primarily nitrate) than anticipated. The concentration of nitrogen in the pond at the time of the first analysis ranged from two to six times the mean influent concentrations. Nonetheless, the experiment was continued and an attempt was made to analyze the transient concentration of nitrate and ammonia and their rate of utilization and to determine the variation of the yield coefficient, Y, with time.

The transient character of presumed limiting nutrients was examined in accordance with the kinetic and material balance concept for nonsteady state reactors. For this analysis the following nonsteady state material balance equation for the rate limiting nutrient was used:

$$\frac{dS_1}{dt} = \frac{1}{\theta}(S_o - S_1) - q\,X_1 \qquad (6\text{-}3)$$

in which the symbols are as previously defined in Chapter II. The term $q\,X_1$ represents the uptake of nutrient by the cells in the reactor and can be written in equivalent form as $(\mu/Y)\,X_1$ because $\mu = Yq$. Neglecting the uptake of nutrients by the cells, that is $q\,X_1 = 0$, Equation 6-3 was integrated between time t_a and time t_b for the nutrient

concentration S_a and S_i. The resulting expression (Equation 6-4) is the theoretical hydraulic washout curve for the nutrient concentration:

$$\ln\left[\frac{S_i - S_a}{S_o - S_i}\right] = \frac{1}{\theta}(t_i - t_a) \quad . \qquad (6\text{-}4)$$

Figures 6-7, 6-8, and 6-9 present a comparison of observed mean concentrations of various forms of inorganic nitrogen observed in pond assays of secondary and tertiary waste water effluents in Assay No. 6, together with washout concentration profiles obtained from Equation 6-4. As noted on each of the three figures the mean concentration of nitrogen, S_o, in the pond influent differed very little between secondary and tertiary effluent. All curves presenting assay data begin at 5 days because the first analysis for nitrogen content was made at 5 days after startup of the assay. For use in computations for the washout curve (Equation 6-4) the nutrient concentrations measured in ponds 3, 4, 7, and 8 were averaged to obtain the best estimate of the concentrations, S_a, at t_a = 5 days. The average values of S_a for the several nutrient forms were as follows:

$NO_3 + NH_3\text{-}N$ = 500 $\mu g/\ell$ (Figure 6-7)

$NH_3\text{-}N$ = 205 $\mu g/\ell$ (Figure 6-8)

$NO_3\text{-}N$ = 295 $\mu g/\ell$ (Figure 6-9) .

Figure 6-7 reports the variation in the concentration of the major inorganic nitrogen constituents ($NH_3 + NO_3$) with time in the ponds receiving secondary and tertiary treated effluents. Also shown is the theoretical "washout" concentration profile assuming a completely mixed reactor and neglecting cellular uptake of nitrogen. First, the assumption of a completely mixed reactor is questionable because the rectangular ponds with considerable wall growth are not sufficiently well mixed to model accurately a completely mixed reactor (CSTR), especially for transient conditions. Second, the calculation of the theoretical "washout" concentration profile neglecting cellular uptake of nitrogen permits graphical estimation of the total nitrogen uptake from the apparent difference between the theoretical concentration profile and the observed data.

Inspection of Figure 6-7 reveals that there was considerably greater uptake of inorganic nitrogen in the pond receiving secondary effluent compared to that receiving tertiary effluent. The difference in nitrogen uptake between the ponds receiving secondary and tertiary effluent is confirmed by the substantial differences in observed growth as shown in Figure 6-10 which is discussed later. From these data it is apparent that there is some substance other than nitrogen that is limiting growth in the tertiary treated effluent pond. One possibility of a growth limiting substance other than nitrogen for the tertiary effluent pond is phosphorus. Analyses of the contents of the pond receiving tertiary effluent indicated a phosphorus content at all times less than 1.0 $\mu g/\ell$ PO_4-P. Unlike with nitrogen, there was no significant increase in phosphorus concentrations in the pond associated with the seed culture addition. It is apparent that the concentration of phosphorus or some other substance removed in the tertiary treatment process could be limiting growth in the tertiary pond. A second possible explanation for the relative low nitrogen uptake in the tertiary treated pond is that there may be some toxic materials picked up in the tertiary treatment process.

Figures 6-8 and 6-9 show the individual ammonia and nitrate concentration profiles with time that make up the total inorganic nitrogen profile shown in Figure 6-7. Inspection of Figure 6-8 reveals that the ammonia disappearance rate in the secondary effluent pond was considerably greater than in the pond receiving tertiary effluent. The ponds being fed effluent obviously are not bacteria free and the uptake of inorganic nitrogen forms can result from free and attached

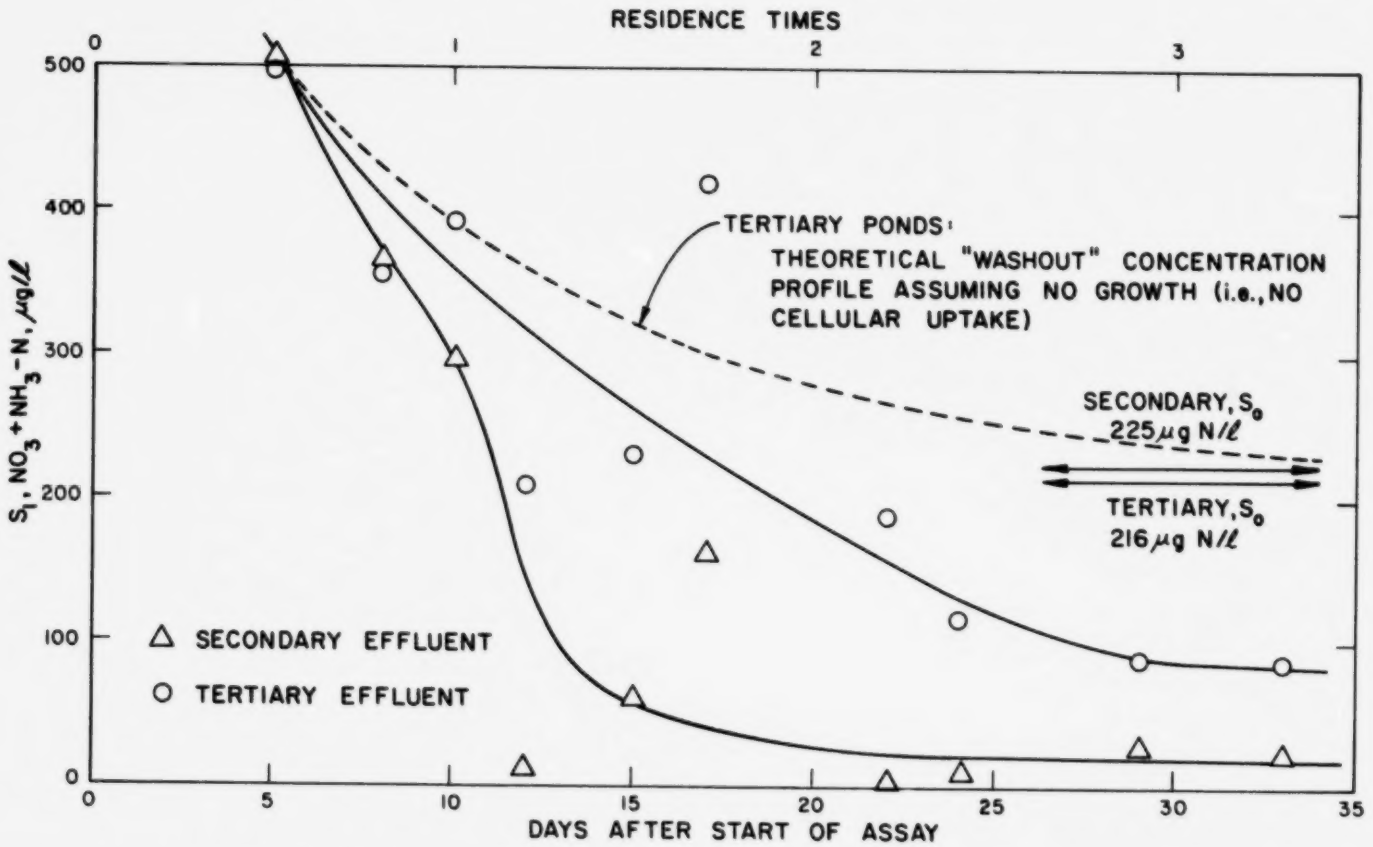


FIGURE 6-7 CONCENTRATION OF INORGANIC NITROGEN DURING POND ASSAY OF EFFLUENTS RECEIVING INITIAL PULSE DOSAGE OF NUTRIENT WITH SEED MATERIAL

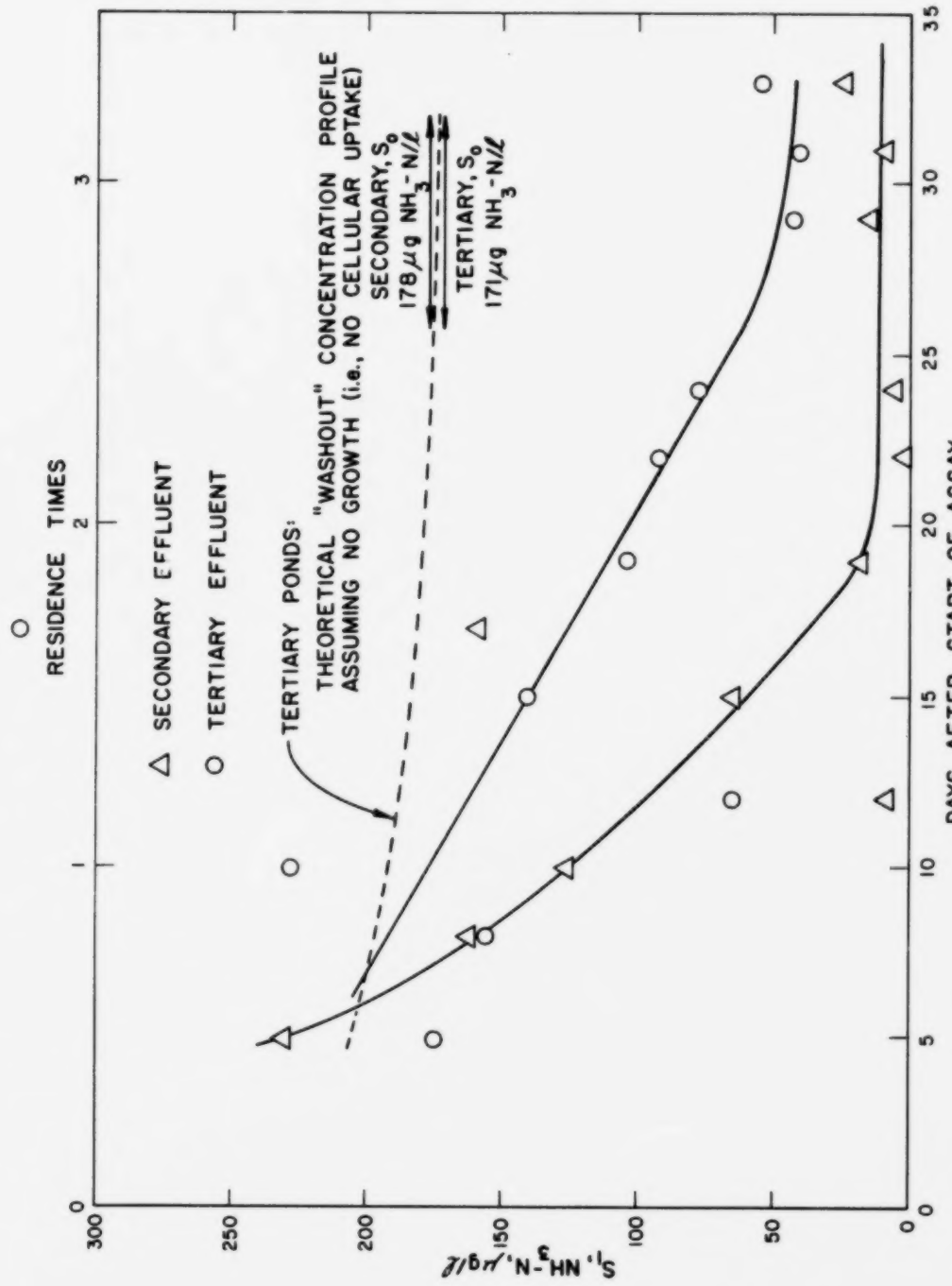


FIGURE 6-8 CONCENTRATION OF AMMONIA NITROGEN DURING POND ASSAY OF EFFLUENTS RECEIVING INITIAL PULSE DOSAGE OF NUTRIENT WITH SEED MATERIAL

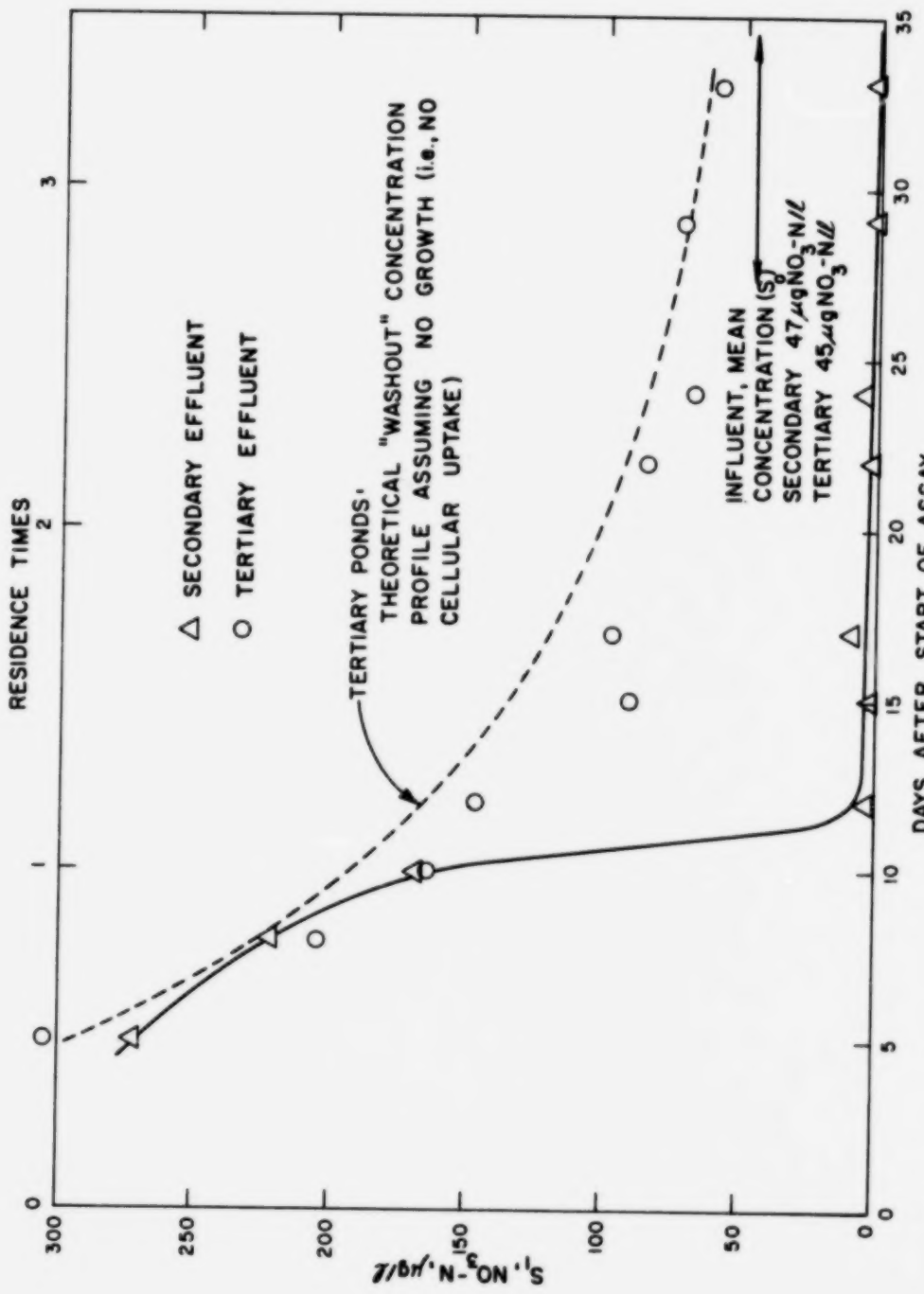


FIGURE 6-9 CONCENTRATION OF NITRATE NITROGEN DURING POND ASSAY OF EFFLUENTS RECEIVING INITIAL PULSE DOSAGE OF NUTRIENT WITH SEED MATERIAL

bacterial growths as well as from algal uptake. This latter point may be significant especially in ponds receiving secondary effluent and for low growth rates, i.e., long hydraulic residence times. However, the data do not indicate the fraction of the ammonia taken up by algal cells, the fraction taken up by bacterial growths, especially those attached to the pond walls or the fraction of the ammonia oxidized to nitrite or nitrate. The oxidized ammonia would most likely appear in the nitrate analysis if it wasn't taken up by the algae. One would expect that the pond receiving a secondary treated effluent would also receive a substantial supply of nitrifying organisms capable of fairly rapid conversion of the ammonia to nitrate. Unfortunately no nitrogen material balances were conducted to assess the degree of nitrification occurring in the ponds. Because of the relatively high pond temperature (15-30°C), the relatively long residence time (θ = 10 days), and the addition of nitrifying flora in the secondary effluent, it seems highly probable that significant nitrification occurs.

As shown in Figure 6-9, the nitrate concentration profile indicates a very rapid disappearance of nitrate in the pond receiving secondary effluent. In fact, the apparent nitrate uptake rate during the early period of the experiment appeared considerably greater than that for ammonia, even assuming that nitrification did not occur. If nitrification did occur then the nitrate uptake rate would be higher than that indicated. Moreover, the nitrate concentration reached a steady state of 2-5 $\mu g/\ell$ NO_3-N in about one residence time whereas with ammonia it took about 2 residence times to reach a steady state level of 20-45 $\mu g/\ell$ NH_3-N. However, part of the reason for the difference in time to reach steady state is due to the difference in the concentration, S_o, of the nitrogen species in the influent. It should be pointed out also that differences in mixing (i.e., incomplete mixing) would make the apparent nitrate uptake rate appear considerably lower than the actual rate whereas the same imperfect mixing would have relatively little effect on the apparent ammonia uptake rate.

For the pond receiving tertiary treated effluent, the nitrate concentration profile appears to follow fairly closely the theoretical "washout" curve with relatively little apparent uptake of nitrate. However, this could result from the composite dynamic effect of nitrate uptake, ammonia oxidation to nitrate and imperfectly mixed ponds. One should be cognizant of the fact that incomplete mixing in the pond would have the greatest effect on the nitrate washout curve tending to reduce the apparent uptake of nitrate. This is true because there is a much greater difference between the peak instantaneous nitrogen species concentration (S_o at t_d = 5 days) and the steady influent concentration, S_o, for nitrate than there is for ammonia. Note the S_o values for each nitrogen species are shown on Figures 6-7, 6-8, and 6-9.

Figure 6-10 shows the biomass (net VSS) concentration profile in Assay No. 6 for the ponds receiving secondary effluent (ponds 7 and 8) and tertiary effluent (ponds 3 and 4). The biomass time concentration profile for pond 1 which had a different history than ponds 7 and 8 with respect to both the addition of seed algae as well as accompanying nutrient addition is also shown. Pond 1 was not seeded and it received only LTW along with secondary effluent at the same concentration as ponds 7 and 8 (1 percent); hence, it did not receive the initial pulse dosage of nutrients or cells.

That there was a marked difference in growth between the 1 percent secondary and tertiary ponds is evident from Figure 6-10. The growth in the tertiary ponds (3 and 4) is relatively insignificant as compared to that occurring in the secondary effluent ponds. Of special interest is the very rapid growth that occurred in the secondary pond which resulted in a maximum biomass concentration of about 6 mg/ℓ before dropping off to a near steady state level of about 2.5 mg/ℓ. This high initial and transient growth rate has been observed previously [1] and it appears to be a characteristic of algal cells placed in a new nutrient-rich environment.

It is apparent from inspection of Figure 6-10 as well as Figures 6-7, 6-8, and 6-9 that a steady state had been reached in all ponds after about 25-30 days or about 2 residence periods. That is, the concentration of biomass (VSS) as well as

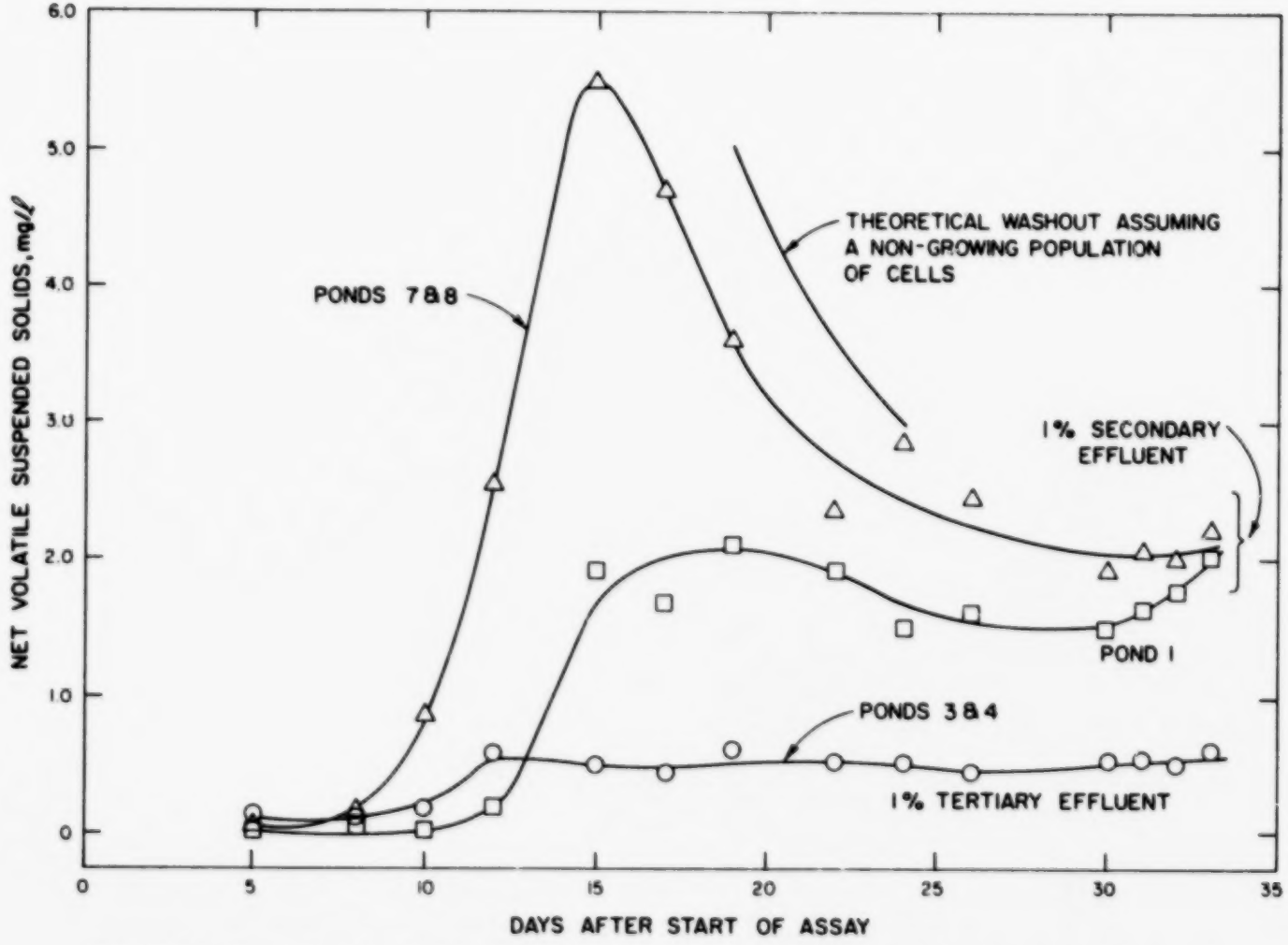


FIGURE 6-10 NET VSS CONCENTRATION DURING POND ASSAY OF EFFLUENTS RECEIVING INITIAL PULSE DOSAGE OF NUTRIENT WITH SEED MATERIAL

nutrients are relatively constant – a condition for steady state. For uniformity in data analysis, an acclimation period of 5 residence times was used for essentially all assays (Assay No. 2 excepted) prior to use of the data for kinetic analysis even though an apparent steady state may have been reached at an earlier time.

Steady State Kinetic Analysis

The large amount of kinetic data obtained in Assays 2 through 7 is reported in Appendices D, E, and F. Analysis of these data is difficult, however, because they involve a wide range of variables and do not define the character of the rate-limiting nutrients. Moreover, many of the critical analyses for the controlling parameters are less accurate and sensitive when applied to outdoor ponds than to laboratory scale units. This is particularly true of cell concentration as measured by volatile suspended solids. Here extraneous (allochthonous) organic noncellular material gaining access to the ponds is reported as VSS, or cells. At the same time wall growth may go unreported; and the lack of thorough mixing may result in a non-representative sample as well as adversely affecting the kinetic model. Moreover, the steady state total nutrient concentration as estimated from the soluble fraction, S_1, and that removed in the cells, γX_1, could account for only 90 percent of the input concentration in 5 residence periods.

In analyzing the transient kinetics of selected assay series it was possible to make corrections for some of the foregoing factors such as the amount of noncellular volatile suspended matter present in the ponds. A correction factor was developed by VSS analysis of samples from a control pond containing only LTW and allochthonous material. By assuming that the allochthonous material was the same in all ponds a "net volatile suspended solids" value was obtained by applying the correction factor to observed values of VSS. Such a correction was applied to all pond data used in the kinetic analysis herein reported.

In evaluating the kinetic analyses which follow it should be borne in mind that the data being analyzed represents only the first and somewhat crude data from the experimental ponds and that more refined and accurate data are expected to come from the next series of assays. It is intended in the "first-cut" kinetic analysis to sort out the data that appears to follow functional concepts; that is, what nutrient or nutrients if any are observed to be growth rate limiting. Once these have been sorted out it will then be possible to conduct more refined experiments to develop the best estimates of the kinetic rate constants and coefficients.

Data Processing Procedures. In selecting data for kinetic analysis as well as in the processing of data a number of simplifying assumptions had to be made to put all of the data in a format for processing with a digital computer. Computer programs were written for the assumed growth-rate limiting nutrient concentration function as well as for the cell continuity equation so that the rate constants and coefficients as well as correlation coefficients for the data could be computed by machine methods.

One of the initial and key definitions that had to be developed was the criteria for steady state. Usually the criteria deal with some variation in either or both the reactor cell concentration or rate-limiting nutrient concentration. Limited experience with the pond reactors indicated that a period of steady state feed of about 5 residence periods was sufficiently long to insure that the variation in the pond cell concentration and nutrient concentration was quite small (within $\pm$ ~ 25 percent of mean value, see Figures 6-7 to 6-10). Thus all data collected after 5 residence period was assumed to be at steady state. It is recognized that this in itself is a crude assumption and needs refinement in future analyses; however, it was believed to be adequate for the first kinetic sorting of the data.

Additional assumptions and definitions made to insure uniform data processing are as follows:

1. Influent nutrient concentrations, S_0, were calculated from analyses of LTW and of the waste effluent, considering the appropriate dilution of the effluent used. Since the concentration of nutrients in the different samples of the effluent differed significantly, this obviously affected the precision of the kinetic analyses. (Data reported in Appendix E.)

2. The pond nutrient concentrations, S_1, were determined for the soluble forms in the ponds. (Data reported in Appendix D.)

3. The cell concentration in the ponds, X_1, was estimated by the volatile suspended solids determination (Appendix F). The cell concentration was estimated by subtracting the reference pond VSS (LTW) to attempt to correct the cell concentration for extraneous materials as well as for growth in LTW.

4. For purposes of data analysis, the presumed rate-limiting nutrient concentrations which were found to be below detectable limits with the analytical method used were assumed to have a nutrient concentration one-half that of the detectable limit.

Kinetic constants and coefficients were calculated for the experimental data in a variety of ways to determine whether there were significant differences between the several experimental assays, as well as to evaluate all of the data collectively by groupings based upon various hypotheses as to the rate-limiting nutrient. In this way it was expected that all of the data collected would be analyzed as to its conformity to the kinetic models hereinafter described, both when constrained by the presumed experimental hypotheses and independent of any predetermined hypotheses. Also, each nutrient determined analytically could be tested as to its possible rate-limiting characteristics. Consequently, it would be expected that for any one set of assays only one nutrient would give reasonable values for the rate constants and coefficients. It is only through the use of machine computation methods that such a broad kinetic model screening procedure is feasible.

The corrected pond data were grouped in the following manner for kinetic constant and coefficient analyses:

1. Assay Nos. 4, 5, 6, and 7, which includes data on θ = 3, 5, and 10 days.
   a. Secondary Effluent Ponds (NH_3 + NO_3-N/Total P ≤ 15) Assume N Limited.
   b. Tertiary Effluent Ponds (NH_3 + NO_3-N/Total P ≥ 15) Assume P Limited.
2. Assay Nos. 5, 6, and 7, which include data on θ = 3, 5, and 10 days.
   a. Secondary Effluent Ponds (NH_3 + NO_3-N/Total P ≤ 15) Assume N Limited.
   b. Tertiary Effluent Ponds (NH_3 + NO_3-N/Total P ≥ 15) Assume P Limited.
3. Assay Nos. 6 and 7, which include data on θ = 5 and 10 days only.
   a. Secondary Effluent Ponds (NH_3 + NO_3-N/Total P ≤ 15) Assume N Limited.
   b. Tertiary Effluent Ponds (NH_3 + NO_3-N/Total P ≥ 15) Assume P Limited.

An additional reason for the above grouping of data for analysis was to check the impression of the investigators that the data from the later (Nos. 6 and 7) assays were more reliable than those from earlier assays.

In general, the hypothesis for the assumed rate-limiting nutrient is as follows. If the NO_3 + NH_3-N/Total P ratio is less than 15, the rate-limiting nutrient is assumed to be nitrogen. If the ratio is greater than 15, the likely rate-limiting nutrient is assumed to be phosphorus. This hypothesis led to the presumption that secondary effluents are likely to be nitrogen-limited and tertiary effluents are likely to be phosphorus-limited.

Kinetic – Cell Materials Balance Equations. The equations used in the statistical analysis of the data resulted from Equations 4 (cell continuity or cell conservation equation) and 5 (kinetic equation) in Chapter II were expressed in two forms; that is, both with and without consideration of a decay term, k_d, in the cell continuity equation.

Including Decay Term, k_d.

$$\frac{1}{\theta_c} = \mu - k_d = Yq - k_d = \frac{Y(S_o - S_1)}{X_1 \theta} - k_d \qquad (6\text{-}10)$$

and

$$\frac{1}{\mu} = \frac{1}{Yq} = \frac{K_s}{\hat{\mu}}\left(\frac{1}{S_1}\right) + \frac{1}{\hat{\mu}} \quad . \qquad (6\text{-}11)$$

Omitting Decay Term, k_d.

$$X_1 = Y(S_o - S_1) \qquad (6\text{-}12)$$

and

$$\frac{1}{\mu} = \theta = \frac{K_s}{\hat{\mu}}\left(\frac{1}{S_1}\right) + \frac{1}{\hat{\mu}} \quad . \qquad (6\text{-}13)$$

A computer program was written to determine the rate constants and coefficients in Equations 6-10, 6-11, 6-12, and 6-13 for each group of assays as described previously for both secondary and tertiary treated effluents considering each of the following presumed and determined nutrients; NO_3-N, NH_3-N, Inorganic-N (NO_3-N + NH_3-N), Total N, PO_4-P, and Total P. Thus a total of 60 sets of rate constants and coefficients were determined, that is, 5 groups of assays for each of the 6 presumed nutrient parameters for ponds receiving both secondary and tertiary treated sewage effluents. Of the 60 sets of coefficients only 14 or about 25 percent of the total, yielded reasonable values (positive values) for both $\hat{\mu}$ and K_s. These sets included only 5 of the presumed six rate limiting nutrients; in no instant did Total P yield positive coefficients when it was considered limiting.

Kinetic Constants and Coefficients. Table 6-8 reports a summary listing of the kinetic constants and coefficients for the 14 sets yielding reasonable (positive) values for $\hat{\mu}$ and K_s. Inspection of the table reveals that of the 5 reported rate limiting nutrient parameters, 5 of the 14 sets of positive coefficients were observed for PO_4-P as rate limiting, 4 showed inorganic nitrogen (NO_3 + NH_3-N) to be rate limiting, 3 showed NH_3-N, and 2 showed NO_3-N as rate limiting nutrients.

The correlation coefficients for each of the equations used in the analysis are also shown in Table 6-8. It is of interest to note that for most of the sets of coefficients, the correlation coefficients are very low indicating poor correlation with the kinetic model and mass conservation equations used. Without question the best conformance to theory was observed for Assays Nos. 5 and 6 for secondary effluent which reported correlation coefficients as high as 0.94 and 0.71 for the two equations

TABLE 6-8

SUMMARY OF MICHAELIS-MENTEN KINETIC MODEL, $\mu = \hat{\mu} S_1/K_s + S_1$, RATE CONSTANTS AND COEFFICIENTS FOR POND REACTORS

Pond Influent	Assay Nos. in Group	No. of Data Points	Presumed Rate Limiting Nutrient	Considering Decay, $k_d = 0$ $1/\theta_c = 1/\theta = \mu - k_d = Y_1 - k_d$						Neglecting Decay, $k_d = 0$ $1/\theta_c = 1/\theta = \mu = Y_1$			
				$\hat{\mu}$ day^{-1}	K_s $\mu g/\ell$	Y mg VSS/mg	k_d day^{-1}	r_1 (k_d)	r_2	$\hat{\mu}$ day^{-1}	K_s $\mu g/\ell$	Y mg VSS/mg	r_2
II[a]	4,5,6	10	NO_3-N[b]	0.042	0.25	16	- 0.151	0.651	0.14	0.11	0.25	42.1	0.14
II	4,5,6	10	Inorg. N	0.572	154.6	2.5	- 0.191	0.30	0.45	2.81	154.6	12.3	0.45
II	4,5,6	10	PO_4-P	0.057	0.47	13	- 0.128	0.78	0.32	0.12	0.47	28.7	0.32
II	4,5,6,7	13	Inorg. N	0.079	10.4	2.6	- 0.184	0.32	0.21	0.58	10.4	13	0.21
II	4,5,6,7	13	PO_4-P	0.053	0.28	13	- 0.135	0.78	0.22	0.17	0.28	31	0.22
II	5,6	7	NO_3-N	0.077	0.018	46	- 0.060	0.74	0.02	0.11	0.018	64.3	0.02
II	5,6	7	NH_3-N	0.016	75.1	0.1	- 0.198	0.02	0.60	1.72	75.1	15.5	0.60
II	5,6	7	PO_4-P	0.126	1.03	35	- 0.044	0.94	0.71	0.16	1.03	44.8	0.71
II	5,6,7	10	NH_3-N	0.005	18.2	0.1	- 0.198	0.02	0.47	0.52	18.2	14.7	0.47
II	5,6,7	10	Inorg. N	0.058	18.6	1.0	- 0.184	0.12	0.29	0.48	18.6	13.3	0.29
II	5,6,7	10	PO_4-P	0.116	0.50	32.5	- 0.057	0.90	0.41	0.16	0.50	45.6	0.41
II	6,7	7	NH_3-N	0.224	0.58	16	+ 0.077	0.62	0.16	0.14	0.58	10.4	0.16
II	6,7	7	PO_4-P	0.065	0.22	25.3	- 0.069	0.75	0.30	0.13	0.22	48.9	0.30
III[c]	6,7	6	Inorg. N	0.013	1.46	0.5	- 0.148	0.40	0.01	1.14	1.46	43.7	0.01

[a]Secondary effluent

[b]Inorg. N = $\Sigma(NH_3 + NO_3)$-N

[c]Tertiary effluent

with PO_4-P as the rate-limiting nutrient. The next best correlation (r_1 = 0.90, r_2 = 0.41) was found for Assays Nos. 5, 6, and 7 with PO_4-P again the rate-limiting nutrient for secondary effluent. The third and fourth best correlations also were found to be for PO_4-P as the rate-limiting nutrient for secondary effluents based upon the Michaelis-Menten model.

Considering only the kinetic equation for the model omitting the decay term, k_d, the highest correlation coefficient observed was 0.71 for PO_4-P as the rate-limiting nutrient; however, the second and third highest coefficients (i.e., r = 0.60 and 0.46) while indicative of relatively poor correlations were observed for NH_3-N as the rate-limiting nutrient.

An especially interesting observation made from the data reported in Table 6-8 is that of the 30 sets of data analyzed for the six nutrient parameters for ponds receiving tertiary treated effluent only 1 yielded positive values of $\hat{\mu}$ and K_s. And this 1 set (Assays Nos. 6 and 7) yielded correlation coefficients of 0.40 and 0.01 which indicates extremely poor correlation with the theoretical model for Inorganic N as the rate-limiting nutrient. The only reasonable conclusions that can be made from these data are as follows:

1. The data are not sufficiently accurate to make possible acceptable correlations.

2. The kinetic and mass conservation models (both with and without a decay term) assuming CSTR ponds do not accurately represent the phenomena occurring in the ponds.

3. Neither phosphate or nitrogen (in any of the species assayed) are significant as rate-limiting nutrients in the tertiary effluents tested.

4. There is some toxic constituent present in the tertiary effluents that affects significantly the kinetic response of algae present in the ponds.

Of the four possible explanations for the kinetic behavior of the tertiary effluents, it is likely that all 4 conclusions are applicable to some degree. However, of the 4, for tertiary treated effluents the indication that some nutrients other than nitrogen or phosphorus are rate-limiting appears to be the most significant and logical explanation of the observed data.

As may be noted from the data shown in Table 6-8, only one set of data (Assays Nos. 6 and 7 for secondary effluent and ammonia as the presumed rate-limiting nutrient) yielded a positive decay rate, k_d, of 0.077 day^{-1}. However, the correlation coefficient was quite low (r = 0.67) indicating considerable scatter in the data. All of the remaining sets (13 out of 14) reported negative decay coefficients, most of which were in the range of - 0.10 to - 0.20 day^{-1}. No adequate explanation is available for the magnitude and occurrence of negative decay rates. According to theory a decay term is required for mass conservation; however, the significance of negative values for the decay rate is not readily apparent.

Table 6-9 presents a comparison of the rate constants and coefficients for the presumed rate-limiting nutrient (PO_4-P) that showed the greatest conformity with kinetic theory (i.e., the highest correlation coefficients). The kinetic constants were computed with the two different forms of the mass continuity equation, both with and without the decay term, k_d.

As can be observed from the data, the magnitude of either the maximum growth rate, $\hat{\mu}$, and the yield coefficient, Y, is slightly affected by the choice of the model used. The magnitude of the decay terms has no apparent effect on the magnitude of K_s. For the data reported herein, the magnitude of the decay term had no substantial effect on the magnitude of the kinetic constants or coefficients.

TABLE 6-9

COMPARISON OF KINETIC RATE CONSTANTS AND COEFFICIENTS FOR SECONDARY EFFLUENTS COMPUTED BY MASS CONSERVATION EQUATION WITH AND WITHOUT DECAY RATE CONSTANT, i.e., $k_d = 0$

Assumed Rate-Limiting Nutrient	Model Used to Compute Coefficient	$\hat{\mu}$ day^{-1}	K_s $\mu g/\ell$	Y mg VSS/mg	k_d day^{-1}
PO_4-P	M-M with k_d	0.05-0.13	0.2-1.0	13-35	- 0.04 to - 0.14
PO_4-P	M-M with $k_d = 0$	0.13-0.16	0.2-1.0	29-49	-

Analysis of the kinetic data for the presumed rate-limiting nutrients and the various groups of assays indicates that for secondary effluents, the only nutrient that yielded growth reasonably in accordance with kinetic theory was phosphate phosphorus (PO_4-P). The PO_4-P data for all groups of assays yielded markedly higher correlation coefficients than those observed for any other nutrient species.

Based upon PO_4-P as the rate-limiting nutrient for secondary effluents, the best estimate of the rate constants and coefficients is shown in Table 6-10.

TABLE 6-10

ESTIMATE OF RATE-LIMITING NUTRIENT AND KINETIC CONSTANTS AND COEFFICIENTS FOR POND REACTORS (Secondary Effluent)

Rate Limiting Nutrient	$\hat{\mu}$ day^{-1}	K_s $\mu g/\ell$	Y mg VSS/mg	k_d day^{-1}
PO_4-P	0.13-0.16	1.0	35-45	0 to - 0.04

Inspection of the constants reported in Table 6-10 indicates extremely low values for the constants and coefficients. The value for K_s of 1.0 $\mu g/\ell$ PO_4-P appears to be unrealistically low. The best estimate of $\hat{\mu}$ of 0.13 to 0.16 day^{-1} is also low; however, not as unrealistic as the value for K_s. The best estimate of the decay term is - 0.04 day^{-1} which is not greatly different from zero on the usual decay rate for bacterial systems of + 0.05 day^{-1}. The yield coefficient (Y) is the most reasonable parameter giving a value of 35-45 mg VSS/mg PO_4-P. The phosphorus content of the cells would be about 2.5 percent which is only slightly more than the upper range of reported values listed previously in Table 4-1.

The kinetic analysis of the data on ponds receiving tertiary treated effluent indicated that there was no significant growth response to any of the species of nitrogen or phosphorus present either individually or collectively. It appears that tertiary effluents are neither phosphorus or nitrogen limited. It is

possible that during tertiary treatment there is sufficient toxicity added to the effluent to obscure the growth-rate limiting nutrient concentration relationship.

There are several plausible explanations for the questionable kinetic rate constants and coefficients obtained from the pond data. It may be desirable to indicate some of the major possible sources of error to alert others to such possibilities as well as to indicate those controllable characteristics that are being considered more fully in the continuing studies. One of the principal sources of error may have been the method of correcting the pond reactor biomass that can be attributed to the diluent for the sewage effluents, that is Lake Tahoe water. Also included in this "correction factor" was allowance for organic debris assumed to be added uniformly to all ponds by the wind since this material appeared in the VSS analysis and was considered as biomass (cells). This correction procedure needs careful scrutiny. A second significant source of error was in determining the presumed rate-limiting nutrient concentrations at the low levels encountered in the reactor. It can be stated that on numerous occasions the difference in computed influent and measured reactor concentration ($S_0 - S_1$) was negative. The influent concentration was computed based upon individual analyses of LTW and the effluents and the relative concentration of each in the pond influent. Other likely significant sources of error include the presence of substantial wall growths on the pond walls, variation in the nutrient or biostimulant concentration in the LTW diluent, and the imperfect mixing or inaccuracy of the CSTR model for the rectangular pond reactor.

Several tentative conclusions can be drawn from the pilot pond reactor assays in full consideration of the preliminary results and the imperfectness of the data. Undoubtedly, the continuing and considerably more refined pilot pond assays will yield better data than that obtained in the initial series of assays. The major conclusions appear to be as follows:

1. Kinetic assays of waste effluents containing a variety of possible nutrient species as well as undefined biostimulants are difficult at best. It appears that a considerable portion of the difficulty stems from the interaction or change in the rate-limiting nutrients, depending upon the relative concentration level of nutrients present.

2. It appears that of all nutrient species present in secondary effluents, the most likely or significant rate-limiting nutrient is phosphate phosphorus. The correlation between the growth rate and PO_4-P concentration was reasonably ($r = 0.94 - 0.71$) good and much superior to that associated with any other nutrient species, individually or in combination. However, the observed rate constants and coefficients are much lower in magnitude than anticipated.

3. For tertiary treated effluents, there was no significant or apparent relation between the growth rate and the various nutrient species investigated. It appears that tertiary effluents are neither nitrogen or phosphorus limited. It is possible that during the tertiary treatment process sufficient toxicity may be added to negate any apparent relation between the growth rate and the nutrient species concentration.

4. Future work with pond reactor systems should include several assays with only the addition of a single presumed rate-limiting species to assess the kinetic constants for the nutrient species under idealized conditions. This would permit comparison of the coefficients for the rate-limiting nutrients alone as contrasted to combination with many other nutrient species to assess the effect of interaction of the several forms of nutrients.

CHAPTER VII

SUMMARY AND EVALUATION OF RESULTS

INTRODUCTION

As stated in Chapter I the objectives of the project herein reported was to determine by the most effective assay method available the potential of various inputs to surface waters to stimulate growth of algae; and to demonstrate the specific meaning of the findings in terms of eutrophication of Lake Tahoe. This meant 1) that the experimental data should give some clue as to which assay method is most effective; 2) that assays should be made of runoff from both developed and undisturbed natural land, as well as of untreated and treated waste waters; 3) that the findings of the assays be evaluated experimentally under lake conditions; and 4) that the relative amounts of growth stimulants from various possible sources in the Tahoe Basin be estimated. Data and interpretations presented in the preceding chapters of this report, and in a First Progress Report [1] dated May 1968, are relevant to all of the foregoing aspects of the study except the final nutrient balance, which is scheduled for a later phase of the study program.

The general procedure, presented in detail in appropriate chapters, was to apply both the batch and continuous flow methods discussed in Chapter II to laboratory assays of various creek waters and waste water effluents, diluted to preselected concentrations with water from Lake Tahoe, and to measure the growth response of a single alga (Selenastrum gracile) under controlled conditions. The results of these assays are presented in Chapter IV (Flask Assays) and Chapter V (Chemostat Assays). Experimental ponds, analagous to large chemostats, were used to test the findings of the laboratory assays under conditions simulating the critical shallow areas of Lake Tahoe. In this case the response of indigenous organisms in terms of increase of biomass was measured, as reported in Chapter VI. The pond assays were only partially completed during the period reported. Further work with these facilities is scheduled for the summer seasons of 1969, 1970, and 1971.

LAKES AS REACTORS

In the plug flow model, or batch reactor, discussed in Chapter II, biochemical reactions take place in a continuously changing environment. In contrast, in the CSTR model the flow is continuous but near steady state conditions persist within the system. Both of these models have been utilized successfully in engineered systems.

In a broad sense lakes may be considered as natural reactors analagous to one or another type of engineered system. In reality, many natural lakes are moderately well mixed, yet contain a very large volume of water in comparison to their natural inflow. The behavior of such a lake might, therefore, be to a reasonable degree subject to simulation by a continuous, plug flow or batch reactor, i.e., a flask culture. Other natural lakes, particularly small ones, are subject to a comparatively large annual throughput of water. Such lakes, although well mixed, have a short residence period and might with reasonable accuracy be simulated by a CSTR model, a continuous flow mixed culture. However, between these extremes, lakes are highly complex systems, hence it must be expected that any laboratory simulation is only an approximation of the real system.

The feasibility of simulating Lake Tahoe by an experimental reactor is difficult to predict on rational grounds. Weighed against the criteria for reactors it exhibits characteristics of both batch and continuous flow systems. For example, it has a theoretical residence time of 700 years. Both vertical mixing in the winter and internal currents throughout the year combine to mix it annually to such a degree

that on a long term basis Lake Tahoe might be assumed to be similar to a reasonably well mixed CSTR. That is, the reactor is well mixed throughout its volume at least 700 times each residence period. This mixing, together with the fact that the annual throughput to the lake is some 7.88×10^9 cubic feet, also suggest that it may behave as a CSTR reactor. Such a possibility is further suggested by present indications that the lake is in near steady state conditions at least with respect to major nutrient concentrations. At the same time, pulses of algal growth, especially in the near shore areas, as well as the relatively short time scale associated with the biological reactions responsible for algal blooms indicate the transient characteristics of batch reactors.

Fortunately for the phase of the study herein reported it is not necessary to simulate Lake Tahoe itself with some type of a reactor, although experimental findings must eventually be related to the lake. The first concern is 1) how the water from the lake might be assayed with any type of a reactor system to determine its response to added nutrients; 2) to apply that system in evaluating such response to the spectrum of nutrient sources in surface waters; and 3) to explore the response of the shallow inshore waters of Lake Tahoe to nutrients found to have a significant stimulating effect. It is to these three aspects that experimental work has been directed. In interpreting the results the underlying rationale is that if the warmer shallow areas of Lake Tahoe, which are the most readily observed by the public, produce algal growth under any controllable concentration of nutrients, it must be presumed that the lake is adversely affected and that controls are necessary regardless of the larger question of how the lake reacts as a whole and at what time the entire lake will exhibit eutrophic characteristics.

ALGAL RESPONSE IN LAKE TAHOE WATER (LTW)

As reported in Chapters IV and V, 75 flask assays and 4 chemostat assays were made of Lake Tahoe water in the laboratory to monitor its ability to support growth of the test alga, S. gracile. Samples were drawn from both a near-shore and a mid-lake station. Statistical analyses of the results show that the precision of the cell counting technique by which algal growth response was determined is relatively low. Nevertheless statistically significant values of growth rates were obtained. From flask assays of near-shore samples the time required for the biomass to double itself was found to range from 1.1 to 2.3 days. Mid-lake samples gave essentially the same results, 0.9 to 2.6 days.

The foregoing values indicate that under highly favorable conditions in the laboratory assay the test alga will double its cell mass in a matter of hours. The principal use of the findings is as a base line value against which to interpret the results of assays of other sources of nutrients when introduced into Lake Tahoe water.

For convenience in comparing the growth response of LTW with and without added nutrients the maximum growth rate was expressed as a reciprocal, i.e., day^{-1} ($\hat{\mu}_1$) rather than in days required to double the biomass. Consequently $\hat{\mu}_1$ represents the maximum rate of increase in biomass during a flask assay. In such terms the observed growth rate in near-shore samples ranged from 0.634 to 0.302 day^{-1}, and mid-lake samples from 0.796 to 0.270 day^{-1} (see Table 7-1).

Because there was no way to predict the best way to measure growth response, to additional values, $\hat{\mu}_{1,\ell}$ and $\hat{X}_5$, were determined routinely in flask assays. These, as defined on page 18 represent, respectively, the slope of the least squares straight line of best fit to the cell count data which defines the log growth curve; and the cell concentration existing in the culture flask at the end of a 5-day period of assay. Thus three measures of growth response were obtained for statistical study to determine which was the most sensitive measure of algal growth resulting from nutrients in LTW. The range of values of $\hat{\mu}_{0,\ell}$ and $\hat{X}_5$ for LTW are included in Table 7-1.

TABLE 7-1

SUMMARY OF GROWTH RESPONSE OF S. gracile IN LAKE TAHOE WATER
AS MEASURED BY GROWTH RATES AND CELL CONCENTRATION
(mean of 5 replicates)

Sampling Location	Range of Growth Rate, $\hat{\mu}_b$ ($days^{-1}$)	Range of Growth Rate, $\hat{\mu}_{b\ell}$ ($days^{-1}$)	Range of Cell Concentration, $\hat{X}_5$ ($cells/mm^3$)
Near-Shore	0.634 - 0.302	0.336 - 0.207	255.8 - 32.6
Mid-Lake	0.769 - 0.270	0.548 - 0.049	276.0 - 62.4

Statistical evidence presented in Chapter IV indicates that:

1. The growth rate, $\hat{\mu}_{b\ell}$, in LTW is statistically most significant.
2. $\hat{\mu}_b$ and $\hat{X}_5$ reflect two different types of response to nutrients in LTW.
3. $\hat{X}_5$ appears to be better than $\hat{\mu}_b$ in measuring the response of S. gracile to LTW.

Considering again that the numerical values in Table 7-1 cannot be directly interpreted in terms of eutrophication, their usefulness as a basis for comparison with other assays is dependent upon both the relative range of values observed in such other assays and the causes of the wide range of values reported in Table 7-1.

The overall fluctuation of data is not fully evident in Table 7-1 because, as shown in Tables 4-4, 4-5, and 4-6 (Chapter IV) the greatest or least values of $\hat{\mu}_b$, $\hat{\mu}_{b\ell}$, and $\hat{X}_5$ do not necessarily derive from the same assay (i.e., $\hat{\mu}_b = 0.634$; $\hat{\mu}_{b\ell} = 0.336$; and $\hat{X}_5 = 276.0$ occurred in different samples). This, however, is not in itself a serious matter because of a difference in what the various measures reflect. It means, however, that the same measure should be applied when comparing two different assays.

The most readily identifiable reasons for the range of values observed for any single measure of growth response appears to be limitations of the cell counting technique and seasonal variation in the nutrient content of LTW. The results of chemical analyses of LTW presented in Appendix B show an apparent wide fluctuation in the composition of LTW which can be attributed in part to the difficulties of making precise measurements of low concentrations of chemicals (e.g., < 1 $\mu g\text{-}N/\ell$; < 1 $\mu g\text{-}P/\ell$), such as prevail in the lake; moreover, that substantial experimental error occurs in making cell counts at the low cell concentration supportable by small amounts of nutrients is well known. Consequently, the data show little correlation between nutrient concentration and the growth response measures, $\hat{\mu}_b$, $\hat{\mu}_{b\ell}$, and $\hat{X}_5$. Although this indicates the need for development of a standard technique capable of reproducible results, it does not render the flask assay incapable of identifying what inputs to surface waters are worthy of testing under Lake Tahoe conditions.

Evidence of seasonal variation in the maximum growth rate and maximum cell concentration of S. gracile in LTW is reported in Chapter IV. Figure 4-2 suggests that the effect is cyclical, with the low point occurring in the month of January. Continuing monitoring of growth response in LTW is expected to clarify this point.

EFFECT OF SURFACE RUNOFF

The effect of surface runoff in stimulating growth of S. gracile in LTW was explored by flask assays of various concentrations of creek waters in LTW. Ward, Incline, and Upper Truckee-Trout creeks were selected as representing, respectively, surface runoff from relatively undeveloped, newly developed, and well-established urbanized land areas.

Assays were made of various concentrations (1%, 10%, 50%, and 100%) of creek waters in LTW. Results are presented in detail in Tables 4-8, 4-9, and 4-10, and in subsequent tables and discussion, of Chapter IV. For a rough comparison of the creeks and LTW, Table 7-2 is presented.

TABLE 7-2

SUMMARY OF GROWTH RESPONSE OF S. gracile IN CREEK WATERS AS MEASURED BY GROWTH RATES AND CELL CONCENTRATION
(mean of 5 replicates)
(100% concentration)

Source of Sample	Range of Growth Rate, $\hat{\mu}_2$ ($days^{-1}$)	Range of Growth Rate, $\hat{\mu}_{2\ell}$ ($days^{-1}$)	Range of Cell Concentration, $\hat{\chi}_5$ ($cells/mm^3$)
Ward Creek	0.926 - 0.192	0.432 - 0.166	290.6 - 57.2
Incline Creek	0.842 - 0.508	0.495 - 0.247	448.4 - 155.0
Upper Truckee-Trout Creek	0.824 - 0.450	0.519 - 0.332	433.6 - 153.2
Lake Tahoe (Near-Shore, Table 7-1)	0.634 - 0.302	0.336 - 0.207	255.8 - 32.6

An inspection of Table 7-2 immediately suggests that Ward Creek and Lake Tahoe were not particularly different in growth response as measured by cell concentration, $\hat{\chi}_5$, which was reported in Chapter IV to be the most consistent measure in the flask assays of creek and LTW samples. In contrast both Incline and Upper Truckee-Trout creeks show appreciably higher cell counts and do not differ significantly one from the other. This observation is borne out by the statistical analyses of Chapter IV which show that these two creeks, draining populated or disturbed areas, differ little in flask assay response; and that Ward Creek is generally lower except for one instance (in November) when the very high value of $\hat{\mu}_2 = 0.926$ was observed. Whether this represented some discharge to the creek, flash runoff, or other incident is not known. However, all three creeks showed higher values of $\hat{\mu}_2$ at that time, suggesting that precipitation in the fall of the year may have brought in nutrients accumulated on the soil surface.

Upper Truckee-Trout Creek and Incline Creek differed significantly during December and January, the former showing large increases in productivity at a time when in other creeks it was either declining or relatively constant. Apparently this was due to discharges to the creek rather than to seasonal factors, although in the absence of an accurate history of discontinuous discharges to the creek this possibility cannot be confirmed.

Statistical assessment of the results of assays of concentrations other than 100% creek water, show that only the 50% and 100% samples showed any detectable difference in growth stimulation. At lower concentrations any differences which may

have existed were overwhelmed by the seasonal variation in LTW and the experimental error inherent in the techniques of assay and analysis. It was shown, however, that all creek waters which showed any significant difference at the 50% concentration as compared to the 100% assay also showed a definite reduction in the growth rate, $\hat{\mu}_b$. On one occasion, dilution of the Upper Truckee-Trout Creek water increased its productivity — a phenomenon that could signify the presence in the sample of some material toxic to the test organism.

When growth responses ($\hat{\mu}_b$, $\hat{\mu}_{1\ell}$, and $\hat{X}_s$) were examined by the flask assay method in relation to initial nutrient concentration, S_o, (see Appendix B, for chemical analyses) it was found (Chapter IV) that a linear relationship appeared for nitrogen in the approximate range of S_o = 100 to 300 μg N/ℓ. Below 100 $\mu g/\ell$ there was little identifiable relationship between growth rate and total nitrogen.

In relation to the enrichment of Lake Tahoe the flask assays support the conclusion that the runoff from the undeveloped areas assayed did not differ appreciably from the lake itself, but that definite enrichment of the lake can come from surface drainage of urban and developed land. Furthermore, for concentrations of nitrogen greater than about 100 $\mu g/\ell$, the growth of S. gracile, at least, is proportional to the amount of nitrogen present. Granting that the resolving power of the flask assay technique used is low, ample evidence was produced to justify demonstration of the biostimulatory effects of surface runoff under lake conditions. Such is one of the objectives of further study with experimental ponds in 1969-70.

EFFECT OF WASTE WATER EFFLUENTS

Plans for the management of waste water effluents in the Lake Tahoe Basin call for export from the basin because, waste water effluents are one of the most common causes of man-generated eutrophication of surface waters. The concept of tertiary treatment of waste water is rapidly emerging, with the South Tahoe Public Utilities District (STPUD) pioneering in nutrient removal. Therefore both need and opportunity exist at Lake Tahoe for study of the growth response of algae to growth stimulants in waste waters subjected to various degrees of treatment.

During the period covered by this report assays were made of untreated sewage and of primary, secondary, and tertiary effluent by both the batch and continuous flow methods. The results of flask and chemostat assays are summarized in Chapters IV and V. Flask assays were confined to samples from the STPUD, whereas both STPUD and Reno-Sparks Treatment Plant effluents were assayed in the chemostat studies.

The presence of growth stimulants in domestic waste water is well established; however a summary of three major assays of STPUD presented in Table 7-3 may serve to give scale to the comparative ability of LTW and diluted effluents to stimulate growth of S. gracile.

From an inspection of the data in Table 7-3 it is evident that in flask assays a 10% concentration of waste water was far more productive of algal growth than was LTW. This is, of course, to be expected. Not so expected is the relatively low ability of raw sewage to support growth of the test alga as reported and assessed statistically in Chapter IV. At the 10% concentration level both primary and secondary effluents supported significantly greater algal growth than did raw sewage. In fact, a 1% concentration of tertiary produced a significantly greater growth response than did either raw, primary, or secondary at the same concentration. These findings suggest that sewage either has some toxic factor which limits the growth of S. gracile or that biostimulants are produced during sewage treatment that are limiting in untreated and primary effluents. However, because secondary effluent also showed this same tendency it is more likely that the depressed growth response of untreated sewage resulted from a toxic factor which is reduced by dilution and biologic activity. Nevertheless, the flask assay data indicate that all types of sewage, including tertiary effluent produce a toxic reaction with the test alga, S. gracile.

TABLE 7-4

SUMMARY OF GROWTH RESPONSE OF S. gracile IN WASTE WATERS AT 10% CONCENTRATION IN LAKE TAHOE WATER AS MEASURED BY GROWTH RATES AND CELL CONCENTRATION
(Flask Assay)
(mean of 5 replicates)

Source of Sample	Range of Growth Rate, $\hat{\mu}_a$ ($days^{-1}$)	Range of Growth Rate, $\hat{\mu}_{a\ell}$ ($days^{-1}$)	Range of Cell Concentration, $\hat{X}_s$ ($cells/mm^3$)
Untreated[a] Sewage	0.92 - 0.77	0.815 - 0.457	1067 - 514
Primary[b] Effluent	1.00 - 0.81	0.725 - 0.624	1074 - 828
Secondary[b] Effluent	0.98 - 0.87	0.912 - 0.582	1435 - 1005
Tertiary[b] Effluent	1.09 - 1.02	0.667 - 0.599	701 - 422
Lake Tahoe (Near-Shore, Table 7-1)	0.634 - 0.302	0.336 - 0.207	255.8 - 32.6

[a]Values based on 3 sampling dates

[b]Values based on 2 sampling dates (phosphate removal only)

As in the case of both LTW and creek waters, statistical analysis of waste water assay results indicated that cell concentration $\hat{X}_s$ may be the best measure of growth response, although in this case both $\hat{\mu}_{a\ell}$ and $\hat{X}_s$ appeared superior to $\hat{\mu}_a$.

Data were too limited to reveal any seasonal variation in growth response of S. gracile in waste waters. Howeve., some relationship between treatment plant loading and growth response appears to exist. It appears that there is greater growth response in waste waters during the period of greatest human activity in the basin. This might be expected as sewage strength normally varies with the season and population in an area such as the Tahoe Basin.

The chemostat assays produced some interesting results indicating apparent toxic-like response affecting the growth rate in various waste waters. For example the steady state cell concentration for Reno-Sparks secondary effluent was 4 times that of a STPUD sample on twice the nitrogen content. Evidence is that growth in the STPUD sample was nitrogen limited, although in flask assays of other samples from this source did not show such limitation. A 10% concentration of Reno-Sparks secondary in one instance required 14 days ($> 2\ \theta$) at a 6-day residence time to reach apparent steady state. In contrast a similar STPUD sample reached an apparent steady state in 2 days ($\sim 1/2\ \theta$) at a residence period of only 4 days. Part of this anomaly might be explained by the initial inoculation being closer to that resulting from steady state growth conditions.

In all successful chemostat assays at equal residence times sewage effluents at any concentration showed a higher cell concentration than LTW, just as did the flask assays. In general, the chemostat assays showed the same type of evidence as

did the flask assays relative to the inputs to Lake Tahoe which should be assayed in experimental ponds.

In the analysis of results of chemostat assays (Chapter V) of sewage effluents assumed to be nitrogen limited, a value of 59 μg N/ℓ was computed for the saturation coefficient, K_s (i.e., concentration of limiting nutrient required for the growth rate to equal one-half the maximum specific growth rate, $\hat{\mu}$). In contrast, for a sample of LTW with nitrogen added in the same amount present in the sewage assay, K_s was only 14 μg N/ℓ even though the maximum specific growth rate, $\hat{\mu}$, exhibited by sewage and added nutrient were the same (0.267 day^{-1}, and 0.265 day^{-1}, respectively).

EFFECT OF PONDED WATER

A few flask assays were made of samples collected from the experimental ponds discussed in Chapter VI, and from Indian Creek Reservoir, which receives the tertiary effluent exported from the STPUD. The results, presented in Chapter IV, are particularly important because of the note of caution they suggest in interpreting the results of assays of water which may have already supported algal growth.

Statistical analysis of the results of flask assays of samples taken from each of 8 ponds on two occasions, showed few differences between ponds containing LTW or secondary or tertiary effluents in LTW. The reason is undoubtedly that the flask assays measured the ability of the diluted waste water to support S. gracile after it had been exposed to an indigenous standing stock in the pond which had reduced the nutrient concentrations to very low levels. This is analogous to situations where samples taken from a lake following an algal bloom have failed to show the presence of nutrients, because they have been assimilated nearly completely by the algal cells composing the pond community. A similar situation may exist in a marina which may have supported a significant algal bloom yet shows little growth potential when a sample is placed in a flask culture.

The Indian Creek Reservoir results showed a somewhat different phenomenon in that on one of the two periods investigated the reservoir water showed a greater growth potential than did tertiary effluent going into the pond some months earlier. The tertiary effluent, however, exhibited definite toxic effects in that at 50% concentration in LTW it supported less growth than at 10% concentration although its nutrient content was greater than that of Tahoe water. Presumably the toxic effect was removed by natural processes active in the reservoir. In a later study the effluent from the reservoir appeared to be phosphorus limited, suggesting that whatever growth the reservoir might support had already taken place.

Particular note is made in Chapter IV that the effluent going into Indian Creek Reservoir prior to October and November 1968 was not as highly treated as that which came later; and that the assays reported are not to be interpreted by the reader as describing the quality of that particular body of water. A more complete investigation is underway currently which is intended to trace the history of the lake (reservoir) formed from highly treated waste water.

ASSAY METHODS

Introduction

As explained in Chapter I, no standard method exists for evaluating the algal growth stimulating characteristics of waste waters. However, biologists have for years used flask cultures for assay purposes and engineered systems employing batch reactors are common in the biochemical industries. Continuous flow reactors are less common in these industries but they have been used extensively in the biological treatment of industrial and domestic waste waters. In recent years algae have been

grown routinely in chemostats and some research has been directed to their use in the measurement of algal growth response.

Chapters IV and V present the results of assays of LTW and various other sources of nutrients by the flask and chemostat methods. Chapter VI presents the preliminary findings of pilot pond type reactor operated as chemostats and analyzed in accordance with kinetic theory. In the course of these assays, and in attempting to interpret the results, particular attention was directed to an evaluation of the method itself. It was not, however, an objective of the project to develop assay methods. Both batch and chemostat type assays were made, largely because the most accurate method of measuring growth stimulating properties was not known and the success of the project depended upon the detection of changes in growth response of algae with changes in concentration of nutrients or other growth limiting factors.

During the period covered by this report the Federal Water Pollution Control Administration initiated a carefully designed experimental program* to develop a standard procedure for measuring algal growth potential and to evaluate the batch and continuous flow culture methods. Leaving to that program the final determination of the most effective method of algal growth assay, the experiences and problems encountered with flask and chemostat assays in the Lake Tahoe study are hereinafter summarized.

Flask Assays

The procedure followed in the flask assays is outlined in Chapter IV. On theoretical grounds the method has been criticized because the organisms grow in a continuously changing environment with a varying age of cells that use different amounts of nutrient in different ways as the environment changes. The question then becomes one of interpretation of results: "who grew how much on what, and when!" On the other hand, it is possible, to analyze statistically the findings of experiments designed to measure various parameters of growth response ($\hat{\mu}_k$, $\hat{\mu}_\ell$, and $\hat{\lambda}_n$) by the flask assay method as well as kinetic coefficients by the chemostat method, all directed to relating the growth rate or response as a function of the rate-limiting nutrient concentration. If the relation between growth response and rate-limiting nutrient concentration is to be determined accurately, it is necessary that other growth limiting factors (energy sources, light, carbon, etc.) be in excess. Otherwise a valid growth nutrient relationship cannot be established.

Figure V-1 shows the effects of carbon limiting conditions upon cell concentration after 8 days of flask culture at low nutrient concentration where only small quantities of carbon were available initially. The decline in cell reproduction after the third day could of course, be attributed partly to nutrient exhaustion. However, in all cases where such a decline occurred, pH values greater than 9.0 occurred in the assay flask, indicating that carbon limiting conditions contributed to the decline in growth response. Thus it is evident that some method of ventilation to insure a free exchange of air containing CO_2 is essential in flask assays.

From a practical viewpoint the flask assay must depend upon the culture of a single species, or a defined mixture of species of algae. Thus, it seems inevitable that the results never can be applied directly to outdoor ecosystems involved in eutrophication. Nevertheless the use of a standard laboratory method should become interpretable in practice, as has been the experience with the widely used BOD_5 test.

Applied to the assay of possible inputs to Lake Tahoe the flask culture method did show consistently that some sources could be expected to increase the productivity of LTW. It also revealed evidence that sewage contains some factor which reduces the growth response to a level less than might be achieved with nutrients alone as the rate limiting factor.

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*Provisional Algal Assay Procedures by Joint Government-Industry Eutrophication Committee on Methods.

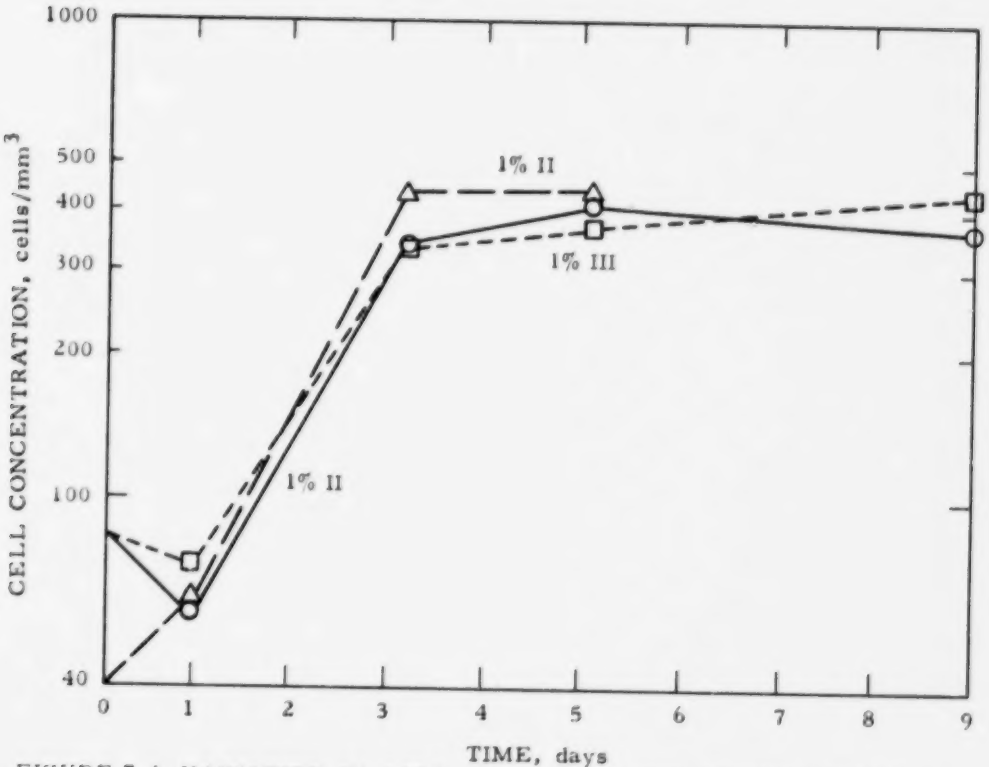


FIGURE 7-1 VARIATION IN CELL CONCENTRATION VS TIME SHOWING EFFECTS OF CARBON LIMITING CONDITIONS AFTER 3 DAYS OF CULTURE

The flask assays also showed that the maximum cell concentration, $\hat{X}_s$, and the growth rate determined by least squares curve fitting, $\hat{\mu}_{cg}$, appears to be more reliable for evaluating growth response than is the observed maximum growth rate, $\hat{\mu}_g$, at least at the frequency of cell counting (1, 3, and 5-day) used in the study.

One of the significant weaknesses of the method for the purposes of this project was the experimental error inherent in the cell counting technique. This reduced the statistical validity of the data and so limited the sensitivity of the method to changes in growth response. Some cell counting procedure is needed which will limit the variance in data more nearly to that inherent in the response of individual organisms to a given environment. The use of a Coulter Counter is planned for future assays at Lake Tahoe. By increasing both the accuracy of single counts, as well as the number of replicate-counts, the variance in counts of cell concentration should be greatly reduced.

Chemostat Assays

The procedure in applying the continuous flow method to the assay of various sources of nutrients at Lake Tahoe is described in Chapter V. From a theoretical viewpoint the continuous flow system has the advantage of operating at a steady state in which the algal cells maintain approximately a constant physiological state. The cell concentration achieved at steady state is then a measure of the growth response to the rate-limiting nutrient. As noted in Chapter II, the kinetic description of a continuous flow system would permit its interpretation in terms of eutrophication in several ways:

1. A given level of rate-limiting nutrient in the receiving water determines the specific growth rate, μ, that can be supported by that concentration of that specific nutrient (S_1).

2. For a given level of S_1 and μ, and yield constant, Y, the mean cell concentration, X_1 (Standing Stock) is determined by the residence time distribution in the system. (See Equation 9, Chapter II.)

3. The net or gross cellular growth rate that can be attributed to any level of nutrient concentration can be estimated once the rate constants and coefficients have been determined for the organism and nutrient species (see Equation 4, Chapter II).

As in the flask assay, some test organism or group of organisms must be used in the chemostat, hence direct application of results to field conditions has similar limitations. However, this limitation seems inherent in any laboratory assay method which might be proposed.

In applying chemostat assays in the investigation several practical problems were encountered and a number of questions raised on the applicability of the method.

First the assay requires considerable time and is more complicated to operate than the flask assay method. Particular difficulty was encountered in achieving and maintaining steady state conditions. Steady state was presumed to prevail when cell concentration on 3 successive days showed reasonable agreement (± 25%). The assay was then continued for 2 additional residence periods, resulting in a total time of from 12 to 20 days to make the assay, in contrast with 5 days for the flask assay. This problem of steady state was particularly acute because of the very low concentration of nutrient in many cases. The critical dilution rate (residence period) for S. gracile was not revealed by the limited range of values observed. However, it appeared that a steady state growth rate could be maintained only in the range of 0.1 to 0.33 day^{-1}. This is much lower than that indicated by any of the flask assays.

As with the flask assays, the difficulty of making accurate cell counts with good precision was encountered. However, the significance of this is considerably greater with the chemostat than with the flask assays because of the considerably lower cell concentrations in the reactor. Consequently the variance in the data is expected to be greater with the chemostats. However, the very fact that lower cell concentrations exist in the reactor than in most of the flask assays is a substantial advantage for the chemostat because it minimizes the possibility of energy limitations (light) which is most likely a factor affecting the cell counts near the end of the flask assay. It is likely that the growth parameters $\hat{\mu}_{\ell}$ and $\hat{\chi}_S$ for enriched samples are affected by light limitations encountered when the cell concentration nears maximum values, often in the range of 500 to 1500 x 10^6 cells/liter at the end of the assay period.

Application of the Michaelis-Menten form of growth – nutrient limiting model (a surface controlled – adsorption type reaction model) and the cell materials balance equation posed some difficulties. First, the question as to what is the rate-limiting nutrient is one that remains to be answered by experiment. Second, assays conducted over a wide range of nutrient levels may include data points where the nutrient level is in excess; that is it has no relation to the observed growth rate of the assay. Third, assays of materials with several possible rate-limiting nutrients may actually experience a change in the rate-limiting nutrient during the conduct of the assay. Lastly, insufficient information is available to date to determine whether or not the cell materials balance equation should include a decay rate term, k_d. Most of the data indicate a negative decay term, for which at present there is no national explanation. Additional reliable experimental data are needed to answer these questions.

In the Tahoe studies, the objectives are to identify the sources of nutrients (in a gross sense) worthy of further study under Lake Tahoe conditions. For this purpose the flask assay method permitted the development of a much greater amount of data on a large number of potential nutrient sources than would have been possible with the chemostat method. Hence the flask assay data appears to be most interpretable for the purpose at hand. However, experience with the two assay methods has been most valuable because in this and subsequent years, the Lake Tahoe evaluation involves the use of pilot ponds operated as large chemostats. It is hoped that the FWPCA - Industry Methods Committee will provide more specific information on the applicability and limitations of the two methods as both that study and this investigation progresses.

Pond Assays

Only the first of a series of pond chemostat type studies planned for the project was completed during the period reported. These involved the assay of waste water effluents. The results are presented and analyzed in detail in Chapter VI.

In conformity with the experimental plan, summarized in Table 6-2 (Chapter VI) a series of assays of secondary and tertiary effluents from the STPUD Waste Water Treatment Plant were made at residence times (θ) of 3, 5, and 10 days. In these assays the ponds were assumed to be CSTR reactors (chemostats) receiving a mix of nutrient species in experimentally determined concentrations and operated at a steady state in which algal growth is balanced by the outflow of cells. Attempts were made initially to utilize the growth response of the test alga S. gracile just as in the flask and chemostat assays in the laboratory. It was found, however, that this organism could not compete with indigenous forms (mostly pennate diatoms), hence the VSS determination replaced cell counts and cell mass as the measure of biomass produced.

The results of 6 pond assays were analyzed for relationship of the mean biomass produced to such environmental factors as light, temperature, residence time, and season as independent variables. The only observed differences as determined by the concentration level of biomass (VSS) found in the pond reactors which were significant at the 95% confidence level were in the assays conducted in June and July (Assays Nos. 2 and 3). These assays had the highest mean water temperatures and incident light; however, they reported the maximum and minimum biomass (VSS) concentrations; namely, 0.91 for θ = 10 days (Assay No. 2) and 0.36 for θ = 5 days (Assay No. 3). Examination of the data from the 6 assays did not reveal any consistent relation with the variables cited; likely because of other variables such as the influence of allochthonous materials introduced into the ponds by wind and rain and seasonal variation in the nutrient content of Lake Tahoe water.

Assays 5 and 6 provided an opportunity to obtain information on growth and nutrient uptake under both transient and steady state conditions. Also a procedure was developed which is explained in Chapter VI for correcting the data to account for both the growth occurring in LTW which was the diluent for all waste effluent pond assays as well as the rain and wind on blown allochthonous material which gained access to the pond reactors. This correction procedure has considerable potential error in that it assumes that all ponds receive the same amount of extraneous organic matter; nonetheless it is the only possible way for rational data analysis.

A graphical analysis was made of selected data (Assays Nos. 4 and 5) on the pond VSS versus the influent concentration, S_o, of nitrogen and phosphorus. For secondary effluents there appeared to be a higher correlation between the biomass concentration and the influent nitrogen concentration than with phosphorus (r = 0.99 and 0.86, respectively). This is what would be expected considering the secondary effluents have a nitrogen to phosphorus ratio of about 2.5 to 1. However, this observation of one assay is contrary to what was observed in the kinetic model data analysis which considered all of the assay data. A similar graphical analysis of biomass versus nutrient concentration for tertiary treated effluents did not indicate any significant correlation with either the influent nitrogen or phosphorus concentration.

In Assay No. 6 the addition of the seed culture produced an unexpected high initial concentration of nitrogen which resulted from nutrient carry over with the seed algae. Consequently, an analysis was made of the transient conditions resulting therefrom. Significantly greater nitrogen uptake rates were observed in the pond receiving secondary effluent than in the pond receiving tertiary effluent. From this observation it appears that the secondary effluent pond might be nitrogen limited; however, such was not the case for the tertiary effluent pond. Also, there was a very rapid initial uptake rate of NO_3-N in the secondary effluent pond; in fact, greater than that for NH_3-N even neglecting the possibility of any oxidation of the ammonia to nitrate in the reactor. If nitrification did take place, the relative NO_3-N uptake rate would be even greater. Unfortunately insufficient data are available to assess adequately the effect of nitrification rates on the uptake of the several nitrogen species. Also the lack of accurate modeling by the pond reactors of a completely mixed pond reactor (CSTR) may affect the interpretation of the transient data.

The steady state data obtained from six different pond assays were grouped in a variety of ways for kinetic constant and coefficient analyses. For the most part the groupings were in accordance with several hypotheses as to the presumed rate-limiting nutrient and the type of effluent (secondary or tertiary) tested. Utilizing digital computation methods it was possible to compute a total of 60 sets of kinetic constants and coefficients by each of two different sets of kinetic models. That is there were 5 sets of kinetic coefficients for each (one at a time) of a total of 6 presumed rate-limiting nutrient species (Total P, PO_4-P, Total N, NH_3-N, NO_3-N and $\Sigma(NO_3$ and $NH_3)$-N) for pond reactors receiving both secondary and tertiary treated effluents. The two sets of kinetic models tested can be described as follows. Both sets used the Michaelis-Menten type (surface controlled reaction model) expression for the growth rate as a function of the presumed rate-limiting nutrient, $\mu = \hat{\mu}\, S_1/K_s + S_1$, and one set used the cell material balance equation, $1/\theta_c = 1/\theta = \mu - k_d$, with a decay rate term $k_d = 0$. The second set used the cell material balance equation with $k_d = 0$.

With the foregoing equations and suitable digital computer programs it was possible to determine the equation of best fit as well as the correlation coefficient and level of significance for each group of data and nutrient species. Obviously, only those nutrient species having some growth rate limiting influence (i.e., relation to growth rate) would give reasonable values for the correlation coefficient and level of significance. Out of the total of 60 sets of kinetic constants and coefficients, only 14 of the 60 yielded positive values for the maximum specific growth rate, $\hat{\mu}$, and the saturation constant, K_s. Of these, 13 were computed for the ponds fed secondary effluent; only one set of positive coefficients was found for the tertiary effluent ponds. Considering the 14 sets of data with positive kinetic coefficients, the highest correlation coefficients ($r_1 = 0.94$, $r_2 = 0.74$) were observed for PO_4-P as the rate-limiting nutrient. In fact 5 of the 15 sets of data showed PO_4-P to be the rate-limiting nutrient and these were found to have consistently the highest correlation coefficients for each group of data and presumed rate-limiting nutrient species.

The best estimate of kinetic constants computed from the pond data for secondary effluents with PO_4-P as the rate-limiting nutrient are as follows:

$$\hat{\mu} = 0.13 - 0.16 \text{ day}^{-1}$$

$$K_s = 1.0 \ \mu g/\ell \ PO_4\text{-P}$$

$$Y = 35 - 45 \text{ mg VSS/mg } PO_4\text{-P}$$

$$k_d = 0.0 \text{ to } -0.04 \text{ day}^{-1} \quad .$$

It is obvious that the observed kinetic coefficients, especially K_s, are very low, however, the data fit the model for these coefficients very well considering the experimental errors involved.

The use of the two sets of kinetic models (i.e., the cell conservation equation with and without the decay term, k_d) had little affect on the magnitude of the coefficients for the data conforming reasonably well with theory. The net effect was to increase slightly the computed constants for the maximum specific growth rate and the yield coefficient (i.e., about 20% for $\hat{\mu}$ and about 40% for Y). However, even these corrections do not make the computed constants and coefficients much closer to that which might be expected. However, note that the yield coefficient Y of about 40 mg VSS/mg PO_4-P is equivalent to a phosphorus content of the cells of about 2.5 percent which is quite reasonable.

The kinetic data for tertiary effluents indicates that neither nitrogen or phosphorus are growth rate limiting.

It is not surprising that the kinetic data from these preliminary assays do not yield more reasonable estimates of the rate constants and coefficients, considering the environmental variations, experimental errors, data corrections and the imperfect simulation of continuously mixed (CSTR) reactors by the rectangular ponds. Chapter VI contains a detailed discussion of these factors. Continuing work with the pond reactors is directed to minimize these errors and it is expected that future data will yield more realistic estimates of kinetic rate constants and coefficients.

ALGAL GROWTH PARAMETERS AND ASSAY METHODS

One of the fundamental questions that must be answered relative to the selection of the best assay method to use for the assessment of the biostimulatory properties of waste is what do the parameters of the method mean and how can they be used in the resolution of practical eutrophication problems. In fact, the identical type of question exists with respect to the selection of the most interpretable or best parameter for a particular assay method. For example, which of the 3 parameters, $\hat{\mu}_1$, $\hat{\mu}_2$, or $\hat{\lambda}_S$ is the best parameter to use in the flask assay method?

Before the question can be answered as to which is the best parameter for a given method, or which assay method is best, considerable attention must be given to the fundamental question: what type of information is desired and how can that information be used? Obviously, also important in the answer to the question is the accuracy and precision of the measurement of the desired information.

In light of the foregoing, it is well to examine the three parameters of the flask assay method as well as the rate constants and coefficients of the chemostat kinetic assay method. In accordance with the most fundamental biological theory, the growth rate of organisms at steady state, and in most instances even in transient state, is some function of the rate-limiting nutrient concentration. With algae, where effort is made to provide limiting factors such as light or carbon in excess, then the rate limiting substance is presumed to be a nutrient source of some type. Inasmuch as all eutrophication problems stem from the growth rate of organisms, it appears quite logical to attempt to determine the function relating growth rate to the rate limiting substance in an environment similar to that found in nature. It is obvious that there are a large number of possible rate-limiting nutrients and that each likely have their own specific function. Considering conditions that occur in nature with a wide range of nutrient combinations and concentrations, it is likely and it appears most probable that there may be a succession of substances each controlling the growth rate for a specific period or range of growth.

Present theory and experimental methods limit the kinetic assessment of mixtures of nutrients to essentially only one rate-limiting nutrient. Therefore, when kinetic studies are conducted it is essential that they be conducted over a limited range of rate-limiting nutrient concentrations. Once experiments are conducted for a specific nutrient when it is in excess and some other material or condition – such as light – becomes rate limiting, then there will be no relationship between the growth rate and the presumed rate-limiting nutrient. This is an extremely important point when setting up experimental designs for determining kinetic constants

on materials such as waste effluents which contain an ample mix of numerous forms and significant concentrations of potential rate-limiting nutrient species.

Determination of the function relating growth rate to nutrient concentration is most difficult with a transient type of assay such as the flask assay. However, estimates can be made with the flask assay of the growth rate as a function of some nutrient concentration parameter. In these and most flask assays, the only nutrient concentration known is the initial concentration, S_o. However, it is possible with the flask assay to determine during the duration of the test the nutrient level as it decreases at the same time the cell concentration, and up to a point, the growth rate is increasing.

Depending on the experimental procedure, which in this case was to make cell counts every 1, 3, and 5 days, the question is what is the best parameter of the growth rate to attempt to correlate with the initial nutrient concentration, S_o. One procedure used was to take the maximum slope of the growth curve, either the 1 to 3 or 3 to 5 day cell count interval and compute the growth rate, $\hat{\mu}_1$. This value, $\hat{\mu}_1$, considers only two data points, and in some cases relatively low cell numbers such as after only one day of incubation where the counting error is substantial. Examination of the flask assay data indicated that for some substrates low in nutrients such as LTW and the Creeks over 75 percent of the samples reported maximum growth rates prior to the 3-5 day period – obviously the 1 to 3 day period. That there is also a concentration and possible toxic factor is indicated by the following data on batch assays of sewage effluents. Thirty-one percent of the samples reported $\hat{\mu}$ for the 1 to 3 day interval at the 50 percent waste concentration level, 46 percent at the 10 percent concentration level, 85 percent at the 1.0 percent concentration and 90 percent at the 0.1 percent concentration level. That an interplay between nutrient concentration and toxic effects exists is apparent in the data. However, the possible effect of light or energy limitations at the higher nutrient concentrations (and cell concentrations) cannot be overlooked.

It should be apparent from the foregoing that one would expect considerable variation in the observed $\hat{\mu}_1$, especially at the lower nutrient and cell concentrations since only two data points are used and these are at the concentration level where the precision of the counting method is minimum. Nonetheless, the computed value of $\hat{\mu}_1$ gives a number that should have a definite relationship to the nutrient concentration.

The parameter $\hat{\mu}_{1,\ell}$ is simply the least squares best fit equation of the log growth curve and as such considers all observed cell concentration data points, 0, 1, 3, and 5 days. It is obvious that such a parameter would have less variation for any given sample than $\hat{\mu}_1$ since it considers all data points; hence the experimental error associated with a single point is evened out. Also the larger variance in the data at low concentrations is averaged out by the higher precision (lower variance) at high cell concentrations such as observed at 3 and especially 5 days incubation. However, there may be an offsetting factor in the parameter. Although the precision of the parameter may be greater than for $\hat{\mu}_1$, it is also possible that at the highest cell concentration (5 days), there may have been substantial change in the growth limiting substance. It seems quite possible that when dealing with cell concentration for $\hat{X}_5$ in the range of 500 to 1500 cells/mm^3 such as for waste effluents, there may have been a change in the assay from nutrient limitation to energy source (light) or carbon limitation. This might mean that the parameter is unduly biased on the low side even though the relative variance of the method is low.

The parameter, $\hat{X}_5$, that is the maximum cell concentration reached at 5 days as defined by practical considerations for this study, is a significantly different type of growth response parameter than either $\hat{\mu}_1$ or $\hat{\mu}_{1,\ell}$. Basically it is not a growth rate parameter but instead only reflects to a variable degree the growth rate. As $\hat{X}_5$ is a cell concentration term at the end of the specific time period of 5 days it is indirectly a rate term. However, it does not directly reflect the growth rate history prior to the fifth day. In fact, in the case of either grossly enriched samples or samples containing considerable toxicity, the test does not measure accurately the biostimulation characteristics of the sample. In the case of the

grossly enriched sample the cell concentration in the assay may increase to such levels that energy (light) limit the maximum population rather than nutrients. In the case of toxicity in the samples it may take a considerable period of acclimatization or adaptation for the test organisms to detoxify the agent or to grow in its presence. Thus, at the end of the 5-day test period the culture may be only at the beginning of the period of rapid or log growth and consequently the 5 day cell concentration level would not accurately reflect the nutrient level of the sample. Also, of the 3 parameters it appears likely that $\hat{X}_5$ is affected to the greatest degree by the possible production of growth stimulating substances by the alga (or associated bacteria if not bacteria free) during the 5 day assay period.

If any of the 3 parameters are to be used in correlation with the dilution of a sample (waste stream, or otherwise) the likelihood of a good correlation with the parameter $\hat{X}_5$ is much higher than if it is to be correlated with specific nutrient species concentrations in the sample. This should be obvious because of the possibility of a succession of rate-limiting nutrients each not responsible for a particularly high rate of growth, but in the aggregate and over the period of the test, producing a much higher cell concentration than could be accounted for by one nutrient alone.

CONCLUSIONS

Because of its simplicity the flask assay method is an economical tool to use in the gross assessment of the relative enrichment characteristics of various waters and wastes. One of the major weaknesses of any bioassay method is the practical interpretation of its findings in any particular problem situation. This is especially true in the typical case of a water which is already productive and needs only an additional enrichment to trigger an explosive bloom of algae. Unless the magnitude of that critical enrichment can be assessed it is impossible to establish the design objectives of engineered processes required to eliminate the danger that a waste discharge will produce disastrous results.

At the present status of knowledge the question of data interpretation is far from answered. In the specific case of the Tahoe studies in which the objective of the initial assays was to identify the sources of nutrient worthy of evaluation under Lake Tahoe conditions, the flask assay method using a specific test alga served as a screening test because qualitative data were the first concern. They revealed several important facts:

1. There is a pronounced cyclical variation in the stimulatory properties of Lake Tahoe water, with the minimum occurring about January.

2. Human activity on a watershed can increase its normal yield of nutrients. Ward Creek has about the same level of biostimulatory properties as Lake Tahoe water, whereas Incline Creek and Upper Truckee-Trout Creek each have about twice the biostimulation of LTW.

3. It was not possible to detect any significant increase in growth response at concentrations of creek water in LTW less than 50 percent.

4. All types of sewage effluents stimulated growth of algae in LTW (see Table 7-3), and it appears that treated effluents are generally more stimulatory than untreated effluent.

Evaluation of sewage effluents in experimental ponds was confined to sewage effluents because it was infeasible to supply the ponds with the large volume of creek water required at 50 percent or less dilution. The ponds were operated as large chemostat (CSTR reactors) and the increase in cell mass of indigenous organisms measured. The results, reported in Chapter VI and discussed in previous sections of this chapter, show that both secondary and tertiary treatment plant effluents at concentrations of 1% and 0.1% in Lake Tahoe water produced greater algal growth than the lake water alone will support. Although kinetic analysis of the data

available at the time of this report are tentative and subject to further analysis when the 1969-1970 data are available, the pond bioassays confirmed the ability of waste water effluents to stimulate growth previously revealed by the flask assays.

To provide the quantitative test data ultimately needed to facilitate control of eutrophication of surface waters it is becoming increasingly apparent that major effort should be directed to relating algal growth rates to specific nutrient concentrations. Specifically, additional work needs to be done to better identify the critical rate limiting nutrient species in the receiving water environment. Evaluation of the constants in the growth rate function through analysis of chemostat data should permit quantitative estimation of the need for or the effect of reducing the level of any specific species of nutrient in the environment, and so identify the measures necessary to control the algal population; or reduce algal blooms to acceptable levels. With specific reference to Lake Tahoe, such quantitative information would reveal whether waste water effluents may ever be treated adequately for retention in the basin; and make it possible to determine the most effective practical measures for land use management with the objective of minimizing the relase of nutrients to the lake.

APPENDIX A

Analytical Procedures

1. pH and alkalinity were determined by Standard Methods [10].

   Total alkalinity expressed as mg/ℓ $CaCO_3$ was determined by potentiometric titration. The pH recorded was the initial pH of the water before the acid was added in the titration.

2. Iron.

   The bathophenanthroline method outlined by Strickland and Parsons [11] was used for determination of total soluble iron, including ferrous and ferric iron, and complex ferric and colloidal ferrous forms.

3. BOD [10].

   BOD determinations were performed on unfiltered samples. Lake Tahoe water was used to dilute waste water samples when necessary. Generally no dilution for stream water was required.

4. COD [10].

   COD determinations were performed on unfiltered samples. Because both dilute water samples and waste water were analyzed, oxidation was accomplished by using 0.25 N, 0.05 N, and 0.025 N $K_2Cr_2O_7$.

5. NO_3 [11].

   NO_3 in water was reduced to NO_2 by passing the sample through a column of cadmium filings. The NO_2 was then determined.

6. NO_2 [11]

   NO_2 was determined by colorimetric reaction using sulphanilamide and naphthylethylenediamine solution.

7. NH_3 [10].

   NH_3 was distilled into a boric acid solution and then nesslerized. Equipment used for the analyses was divided into two sets, one for distillation of samples with relatively high concentrations of ammonia and the other for samples with low levels such as lake and creek samples.

8. Soluble Organic Nitrogen [10,11].

   The sample remaining after NH_3 distillation (usually about 25 mℓ) was digested with a sulfuric acid-selenium dioxide mixture. When the digestion was complete, the residue was diluted with NH_3-free water, made alkaline, and distilled for the NH_3 as reported above.

9. Inorganic Phosphorus (Reactive) [11].

   The sample was mixed with a reagent containing sulfuric acid, ammonium molybdate, and antimony potassium tartrate, adding afterwards ascorbic acid dissolved in ethyl alcohol. The blue color was then read directly from a Beckman spectrophotometer in a 1-cm or 5-cm cell. (The modification used

was reported by Richard Armstrong, Institute of Ecology, University of California, Davis.)

10. Soluble Organic Phosphorus [10,11].

Organic phosphorus was converted to inorganic by hydrolysis with a strong solution of sulfuric and nitric acids. A 100 mℓ sample with the acid solution was autoclaved, cooled and then made alkaline. The inorganic phosphorus was then determined as described above.

11. Chlorides [10].

Chlorides were titrated with mercuric nitrate.

12. Calcium [10].

Calcium determinations were made by titration with EDTA (ethylenediaminetetraacetic acid).

13. Conductivity [10].

Specific conductance (μ mho/cm) measurements were done with a conductivity bridge, Model RC 16B2, made by Industrial Instruments, Inc.

Precision of Analysis

All laboratory chemical determinations were subjected to replicate analyses on aliquots of the same sample to determine the precision of results attainable by the project staff by the chemical procedure used. Tables A-1 and A-2 present summaries of the statistical analyses on each determination for the years 1967-68 and 1968-69, respectively. They show that with the exception of organic nitrogen and total phosphorus in Lake Tahoe water, where concentrations are extremely small, the chemical analytical work is of good accuracy in terms of the coefficient of variation, C_v.

Table A-2 also presents a statistical analysis of the two methods of filtration cited in Chapter III (0.45 μ HA Millipore and GF/C Whatman glass fiber filter paper). The table shows that there is no essential difference in the accuracy of the two methods.

TABLE A-1

PRECISION OF CHEMICAL DETERMINATIONS
1967-68

Sample	Number of Replicates	Range	Mean $\bar{X}$	Standard Deviation σ	Coefficient of Variation $C_v = \sigma/\bar{X}/\%$
Alkalinity, mg/ℓ, $CaCO_3$	5	38.0 - 38.2	38.08	0.75	2.26
Conductivity, μmho/cm	5	69.8 - 70.0	69.88	0.44	0.47
Chloride, mg Cl/ℓ	5	4.6 - 4.8	4.7	0.09	1.84
Calcium, mg Ca^{++}/ℓ	5	8.4 - 10.0	9.36	0.60	6.39
Iron, μg Fe/ℓ	5	146 - 152	149.2	2.04	9.81
BOD, mg/ℓ	8	66.6 - 73.2	69.51	2.41	3.46[a]
COD, mg/ℓ	5	46 - 54	50	3.16	6.32
NO_3-N, μg N/ℓ	5	64 - 79	70.8	6.47	9.00
NO_2-N, μg N/ℓ	5	12 - 13	12.8	0.40	3.15
NH_3-N, μg N/ℓ	5	15,200 - 17,400	16,040	926.50	5.78
Soluble Organic Nitrogen, μg N/ℓ	5	580 - 865	737	99.78	13.54
Soluble Inorganic PO_4, μg P/ℓ	5	5,520 - 5,840	5,650	110.00	1.95
Total Soluble PO_4, μg P/ℓ	5	5,580 - 5,820	5,748	86.23	1.50

[a]Taken from Table 3-4, pg. 37, of First Progress Report [1].

TABLE A-2

PRECISION OF CHEMICAL DETERMINATIONS AND FILTRATION METHODS

1968-69

Analysis	Sample	(Date)	Filtration Method			
			0.45 μ		GF/C	
			$\bar{X}$	C_V	$\bar{X}$	C_V
Alkalinity mg/ℓ as $CaCO_3$	Truckee-Trout	(3- 4-69)	33.08	2.26	-	-
Conductivity μmhos/cm	Truckee-Trout	(3- 4-69)	69.68	0.47	-	-
Chloride mg/ℓ	Truckee-Trout	(3- 4-69)	4.7	1.37	-	-
Calcium mg/ℓ	Truckee-Trout	(3- 4-69)	9.36	6.39	-	-
Iron μg/ℓ as Fe	Truckee-Trout	(3- 4-69)	149.2	9.81	-	-
NO_2-N μg N/ℓ	STPUD II	(1-18-69)	14.6	12.79	12.8	3.13
NO_3-N μg N/ℓ	Shore LT	(1- 4-69)	4.00	15.75	4.20	17.86
NO_3-N μg N/ℓ	STPUD II	(1-18-69)	40.00	15.52	58.00	10.74
NH_3-N μg N/ℓ	STPUD II	(1-18-69)	15,520.	8.19	16,040.	5.78
Organic N μg N/ℓ	Shore LT	(1- 4-69)	68.6	54.30	-	-
Organic N μg N/ℓ	STPUD II	(1-18-69)	822.	3.68	737.	13.54
Total P μg P/ℓ	Shore LT	(1- 4-69)	2.2	109.09	3.4	86.47
Total P μg P/ℓ	STPUD II	(1-18-69)	5,796.	0.55	5,748.	1.50
Inorganic PO_4 μg P/ℓ	STPUD II	(1-18-69)	5,650.	1.95	5,328.	2.89
Inorganic PO_4 μg P/ℓ	Shore LT	(1- 4-69)	< 1	-	< 1	-

APPENDIX B

TABLE D-1

RESULTS OF CHEMICAL ANALYSES OF SNOW AND MELT-WATER (SW) SAMPLES

Date	Sample	Chemical analysis, mg/l (as element)														
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	$SO_4^{=}$	$CO_3^{=}$	HCO_3^{-}	pH	Cond.	SiO_2 + Al_2O_3	Fe^{+++}	Pb	DOC	COD
[illegible]	[illegible]	[illegible]														
[illegible]	SW1	[illegible]														
	Duplicate	[illegible]														
[illegible]	SW2	[illegible]														
	Duplicate	[illegible]														
[illegible]	SW3	[illegible]														
	Duplicate	[illegible]														
[illegible]	SW4	[illegible]														
	Duplicate	[illegible]														
[illegible]	SW5	[illegible]														
	Duplicate	[illegible]														
[illegible]	SW6	[illegible]														
	Duplicate	[illegible]														

TABLE D-2

RESULTS OF CHEMICAL ANALYSES OF SNOW WATER SAMPLES

Date	Sample	Chemical analysis, mg/l (as element)														
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	$SO_4^{=}$	$CO_3^{=}$	HCO_3^{-}	pH	Cond.	SiO_2 + Al_2O_3	Fe^{+++}	Pb	DOC	COD
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														
[illegible]	[illegible]	[illegible]														

TABLE 5-7

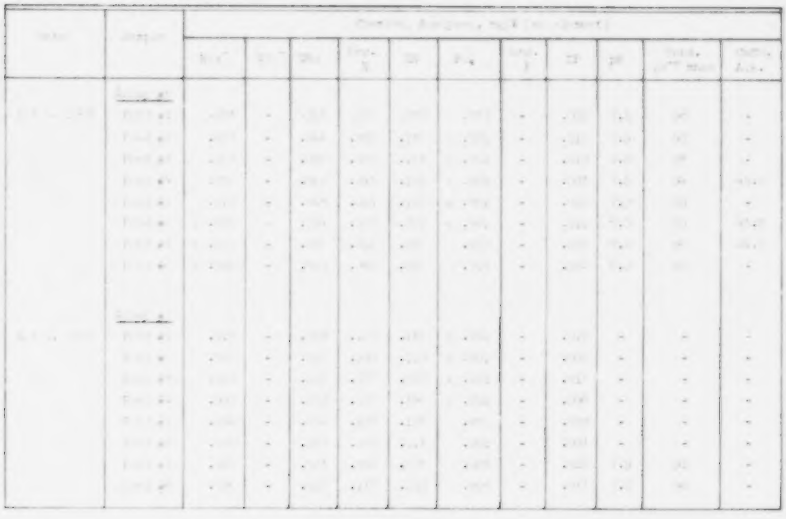
RESULTS OF CHEMICAL ANALYSES ON WASTE WATER EFFLUENTS

Date	Sample	Chemical Analyses, ppm (as indicated)														
		NH_4^+	NO_3^-	NO_2^-	Org. N	TKN	PO_4	Org. P	TP	pH	Cond.	$CaCO_3$ Alk.	Cl^-	Tr.	MBT	CCE
May 21, 1969	II	1.60	.10	4.4	1.4	3.40	1.30	.70	2.0	6	4.4	1740	21.5	.027	47	8
	III	.40	.40	5.9	1.40	13.00	1.40	.60	2.0	7.05	455	245.0	59.5	< .025	< 1	(8)
June 11, 1969	II	1.75	1.40	3.2	1.30	14.45	4.20	.70	4.9	7.1	465	215.0	4.4	< .025	2	11
	III	1.40	1.40	4.7	.55	13.05	1.40	< .05	1.4	7.1	540	180.0	8.4	< .025	< 1	3
June 25, 1969	Raw	.34	4.0	< .05	4.07	4.40	5.5	3.75	9.2	7.4	880	190.0	13.0	.075	140	112
	I	.20	.085	10.2	4.	14.30	4.20	4.80	4.75	7.3	870	184.0	4.4	.050	10	14
	II	5.04	.4	4.5	1.40	11.40	4.4	2.47	5.03	7.3	590	158.	4.4	.038	1	39
	III	7.1	.42	11.80	1.42	20.42	4.6	2.7	4.48	7.3	715	204.	10.4	< .025	1	48
Aug 7, 1969	Raw	< .05	.034	< .1	4.2	< 4.40	2.45	2.4	4.85	7.4	830	214.	14.8	.210	110	164
	I	.085	.008	< .1	3.4	3.4	2.40	1.45	3.85	7.4	830	214.	14.8	.240	110	164
	II	11.41	.47	14.3	1.43	25.43	4.4	2.75	7.15	7.4	715	204.	14.8	.340	10	114
	III	7.9	1.60	15.5	1.41	24.91	3.47	2.05	5.48	7.4	640	170.4	12.8	.210	4	87
Oct 7, 1969	Raw	.125	.090	15.4	1.75	17.4	5.	2.4	7.4	7.4	930	210.0	8.	.430	31	100
	I	.021	.005	1.43	4.05	47.05	4.2	2.45	4.65	7.4	930	210.0	8.	.430	31	100
	II	.051	.015	14.3	1.55	17.4	4.2	2.41	4.6	7.4	715	210.0	8.	.430	31	100
	III	.071	.04	14.8	1.49	16.45	4.49	2.45	4.94	7.4	715	210.0	8.	.430	31	100
Nov 25, 1969	Raw	< .05	.007	15.45	1.45	17.45	4.55	2.4	4.95	7.4	830	214.	14.8	.210	110	164
	I	< .005	.004	15.40	1.45	17.45	4.11	2.41	4.51	7.4	830	214.	14.8	0.37	-	110
	II	.054	.021	15.45	.97	17.41	4.51	2.4	4.91	7.4	715	204.	14.8	0.370	-	94
	III	.045	.02	15.4	.47	15.87	4.36	2.4	4.76	7.4	715	204.	14.8	.410	-	94

TABLE 5-8

RESULTS OF CHEMICAL ANALYSES ON INDIAN CREEK RESERVOIR WATER SAMPLES

Date	Sample	Chemical Analyses, ppm (as indicated)														
		NH_4^+	NO_3^-	NO_2^-	Org. N	TKN	PO_4	Org. P	TP	pH	Cond.	$CaCO_3$ Alk.	Cl^-	Tr.	MBT	CCE
Oct 3, 1969	Dam (A)	.064	.07	.4	.84	.98	.064	.13	.403	7.4	715	214	14	< .025	12	8
	Outlet (B)	.47	1.0	.4	1.47	2.4	.064	.41	.483	7.1	640	170	12	< .025	8	12
Nov 6, 1969	Dam (A)	.08	.05	15.5	1.41	15.61	.141	.061	.211	7.4	640	170	12	< .025	8	12
	Outlet (B)	.04	1.14	15.45	1.45	17.09	.141	.201	.41	7.4	640	170	12	< .025	8	12



APPENDIX C

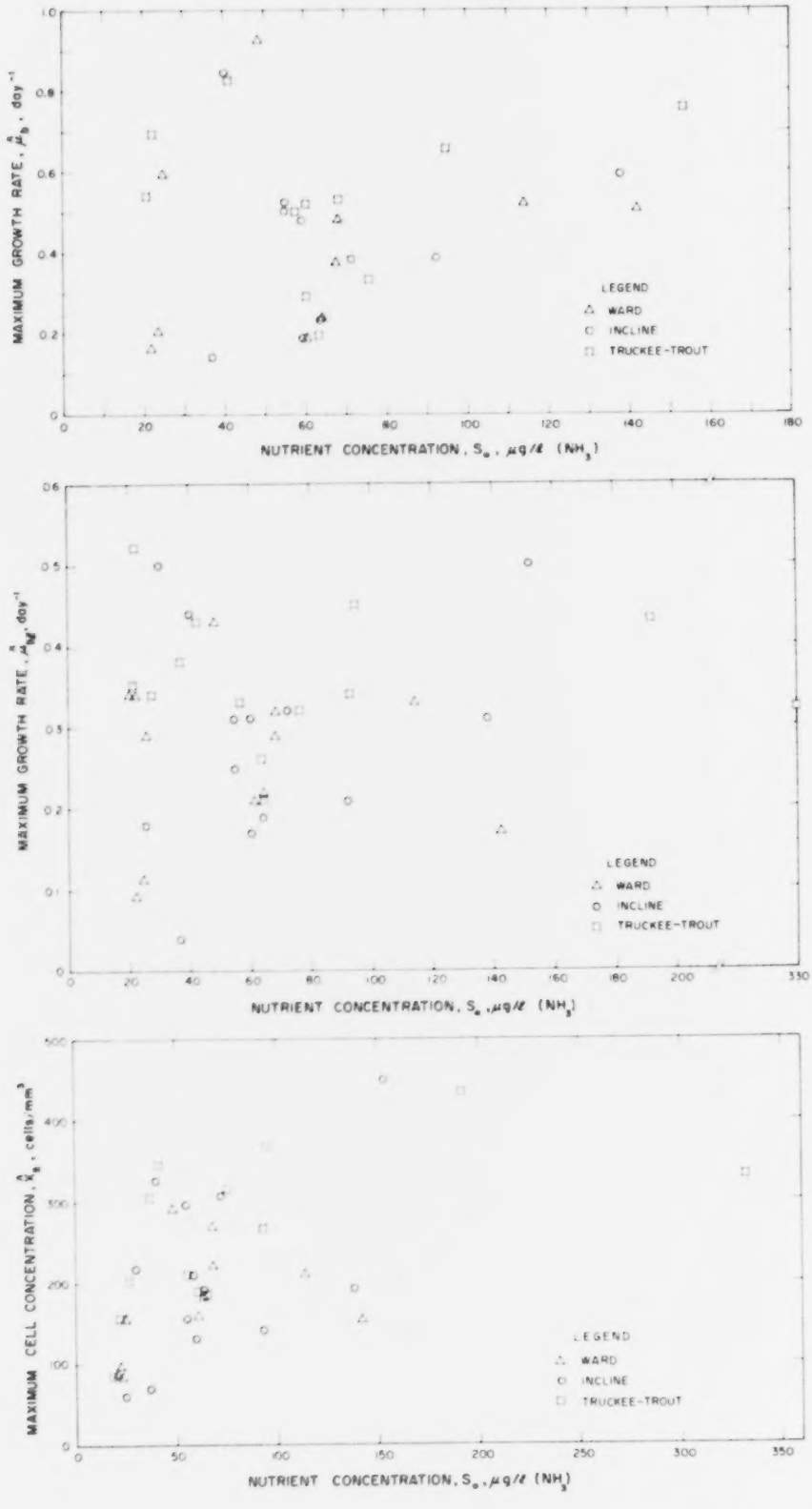


FIGURE C-1 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{NH_3}$, AND CELL CONCENTRATIONS, $\hat{X}_b$, WITH CONCENTRATION OF AMMONIA NITROGEN IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT

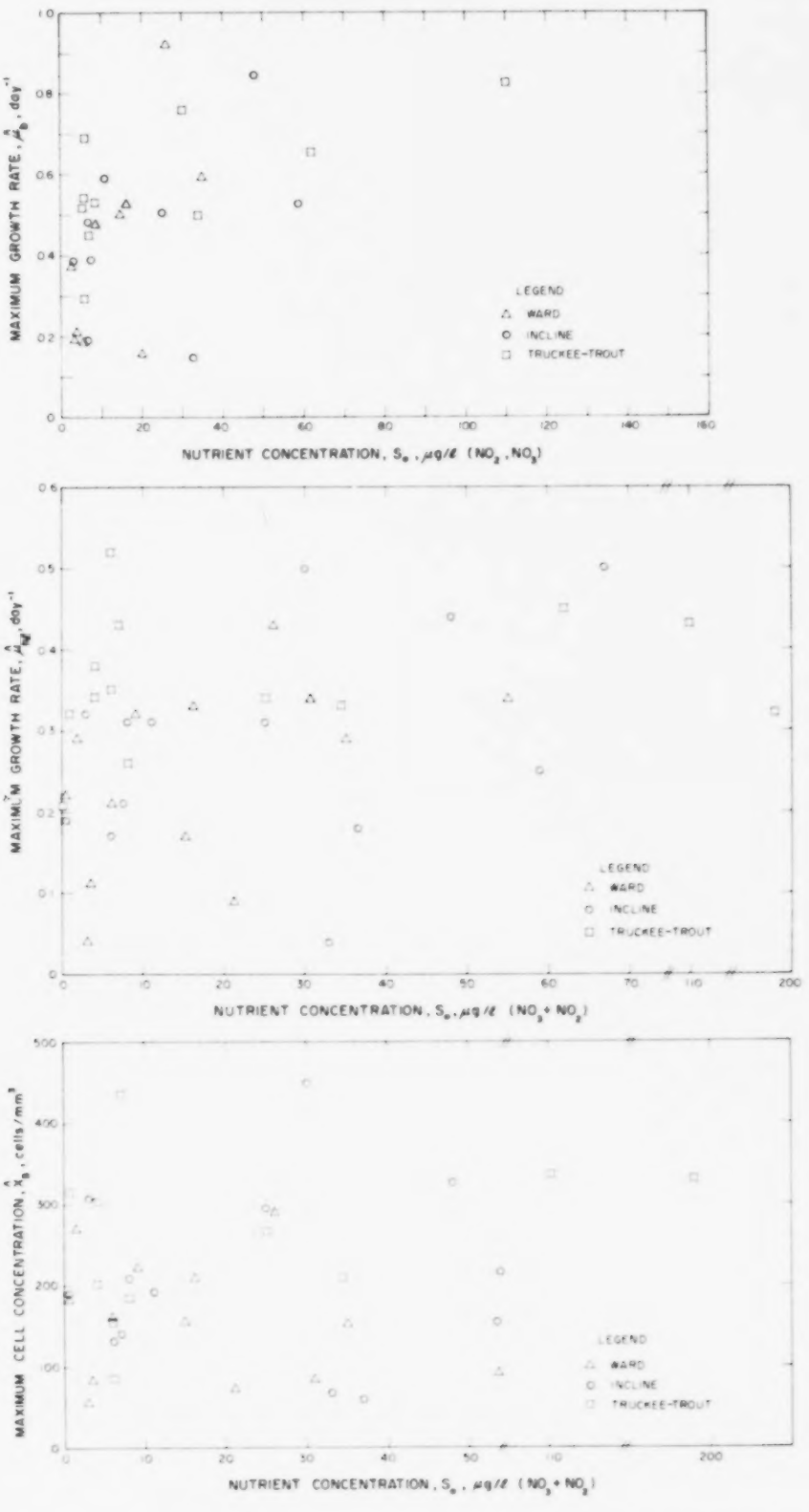


FIGURE C-2 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_0$ AND $\hat{\mu}_{N_2}$, AND CELL CONCENTRATIONS, $\hat{X}_0$, WITH CONCENTRATIONS WITH NITRITE AND NITRATE NITROGEN IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT

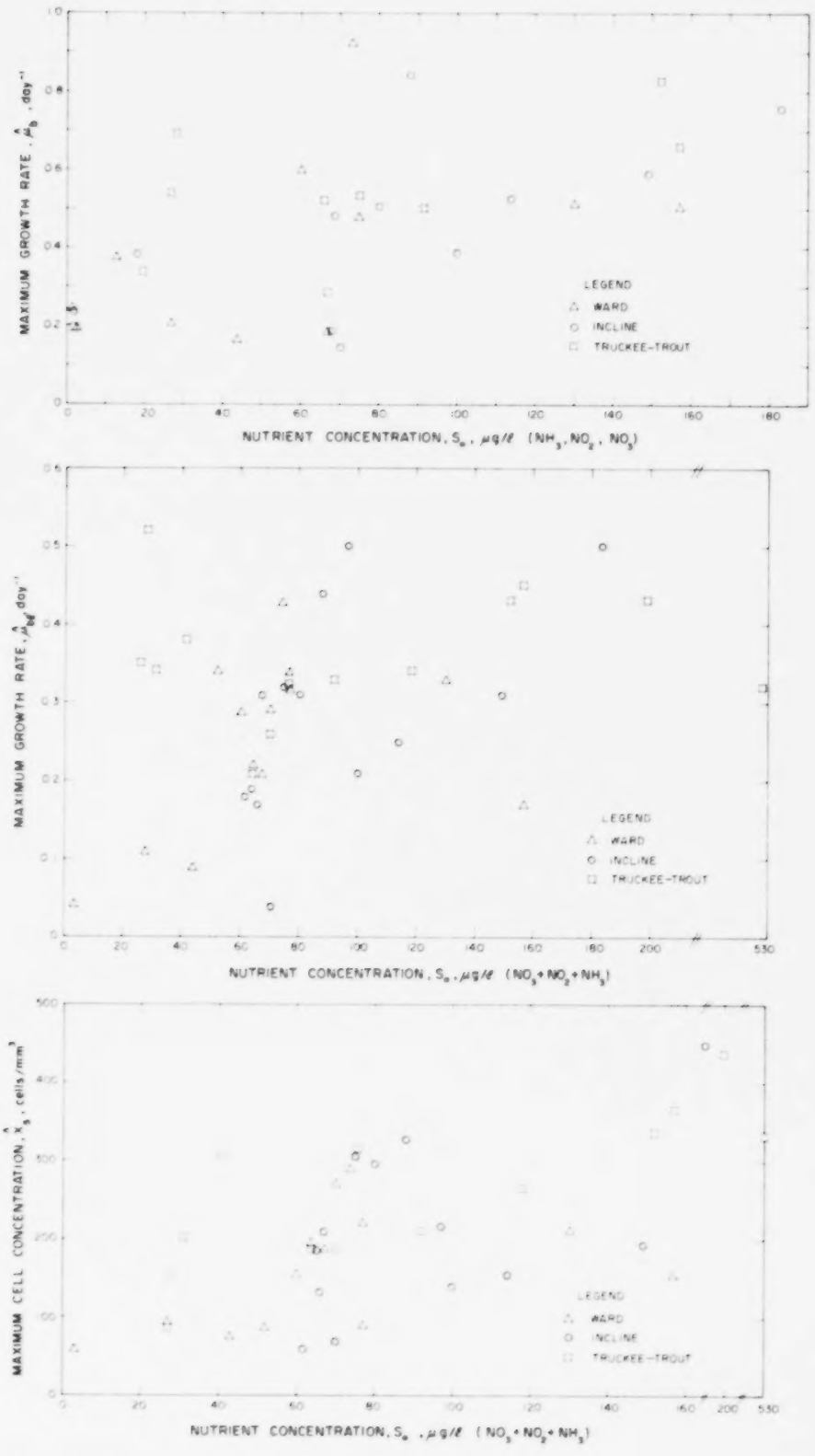
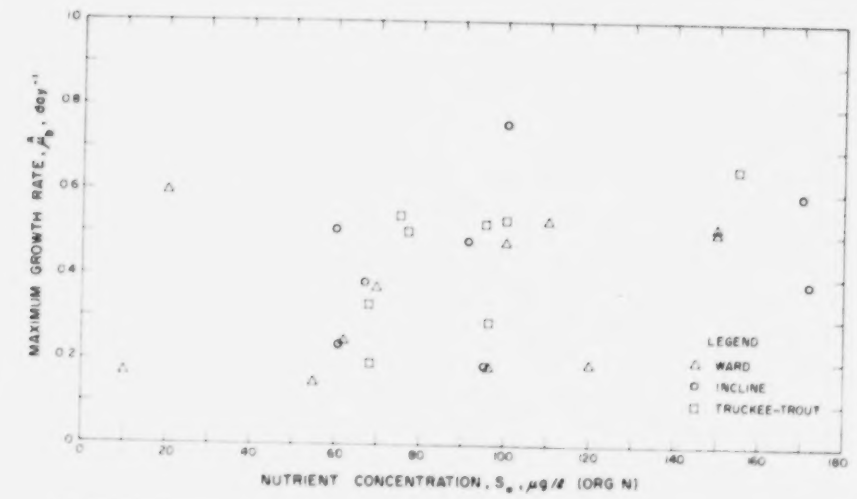
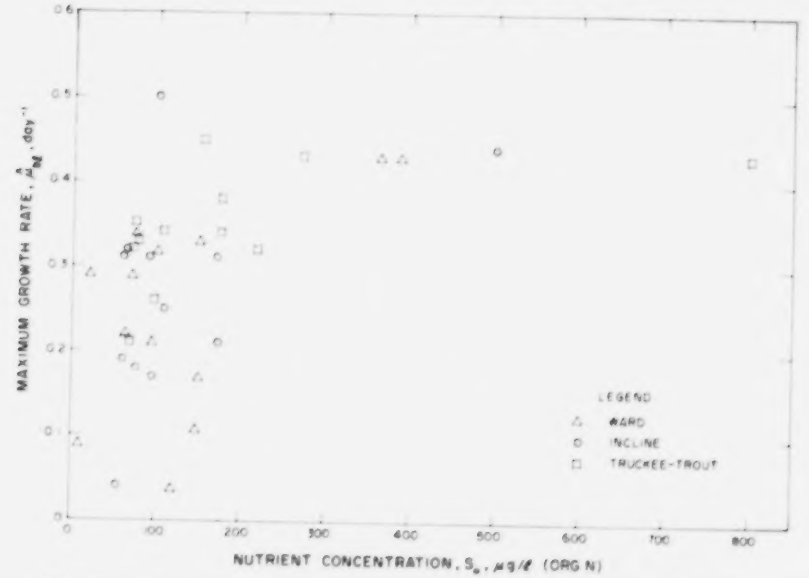


FIGURE C-1 VARIATION OF MAXIMUM GROWTH RATES $\hat{\mu}_b$ AND $\hat{\mu}_M$, AND CELL CONCENTRATIONS, $\hat{x}_s$, WITH CONCENTRATIONS OF AMMONIA, NITRITE, AND NITRATE NITROGEN IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT





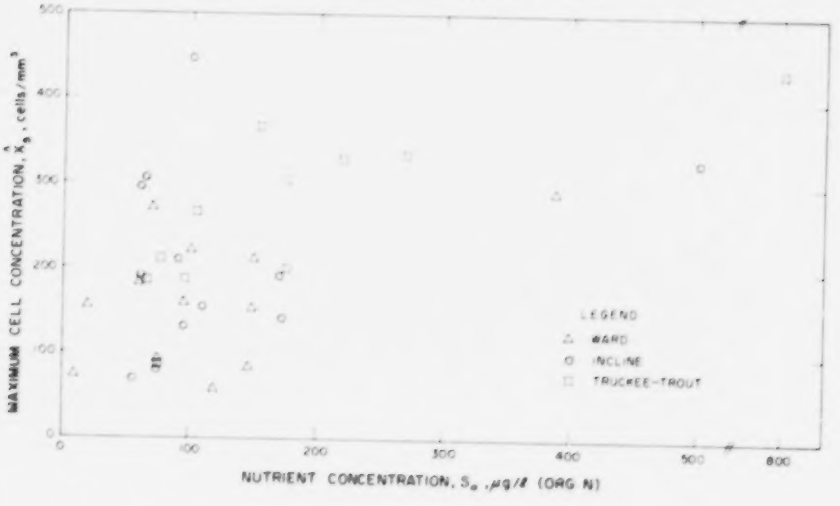
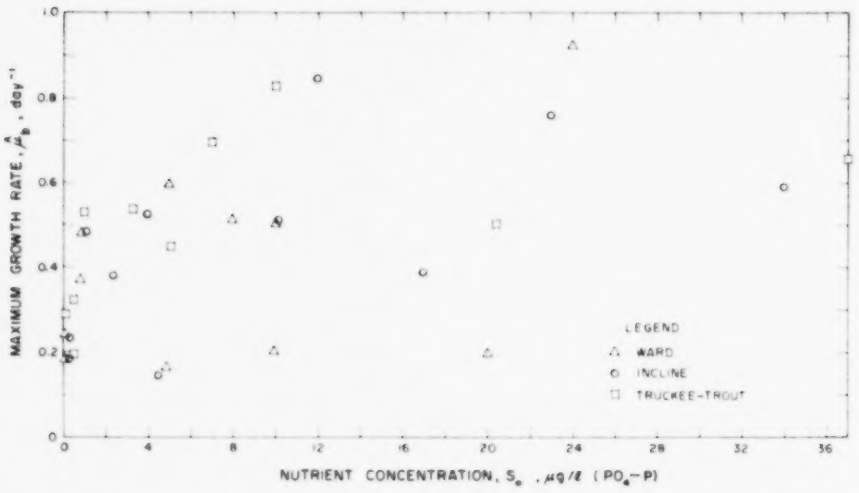
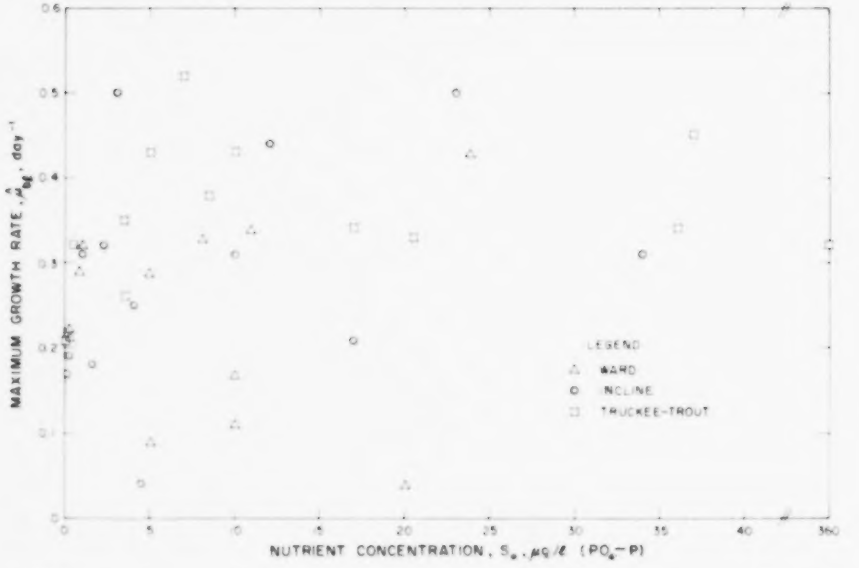


FIGURE C-4 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_N$, AND CELL CONCENTRATIONS, $\hat{X}_s$, WITH CONCENTRATION OF ORGANIC NITROGEN IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT





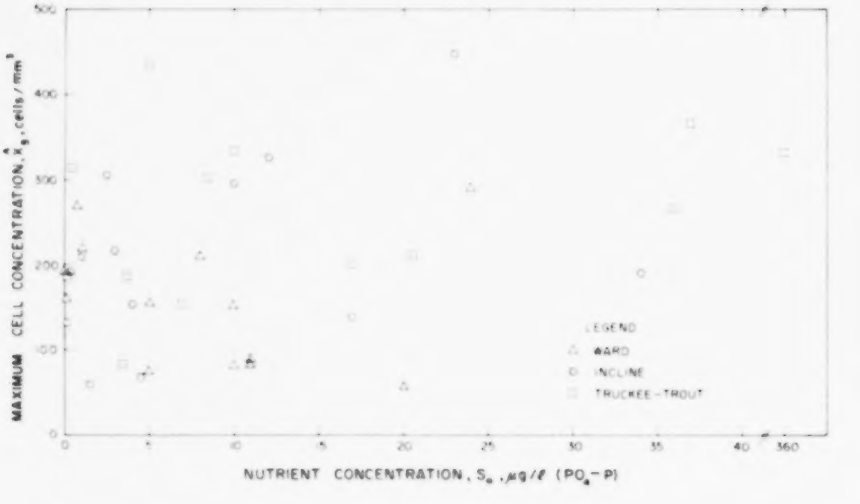
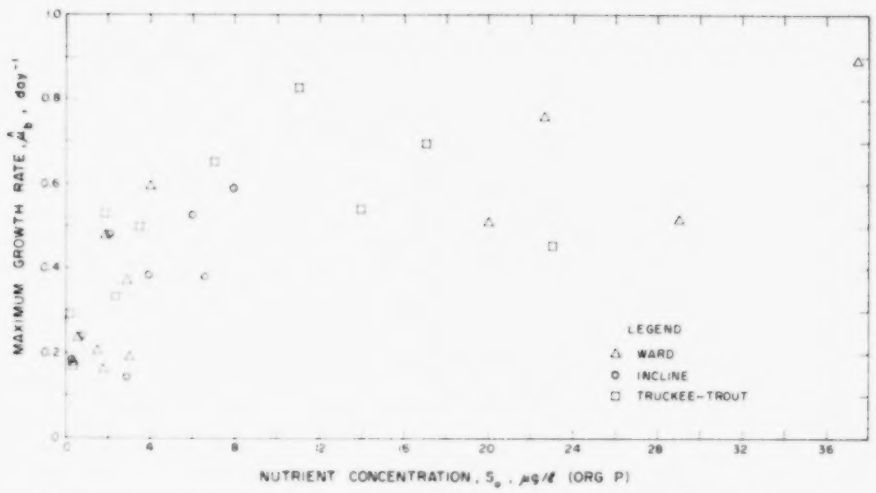
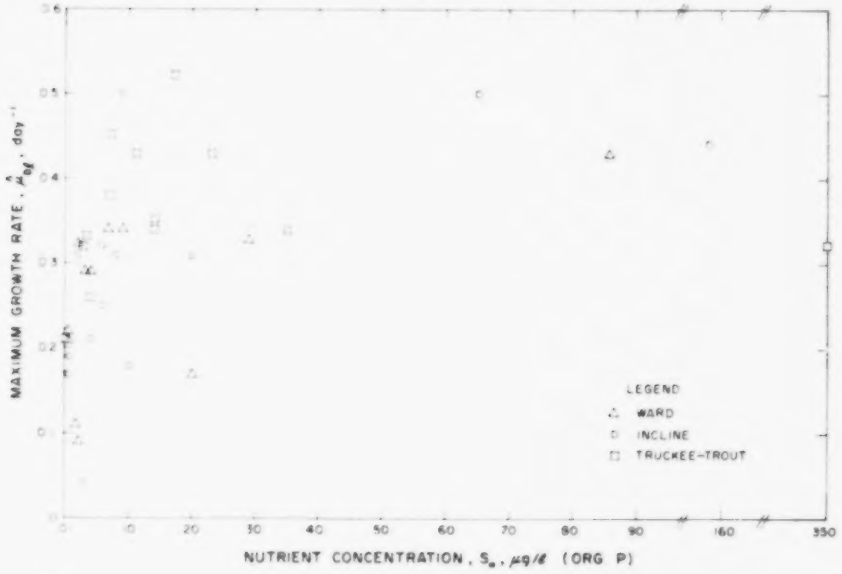


FIGURE C-4 VARIATION OF MAXIMUM GROWTH RATES $\hat{\mu}_b$ AND $\hat{\mu}_{bf}$, AND CELL CONCENTRATIONS, $\hat{X}_b$, WITH CONCENTRATION OF PHOSPHATE AS PHOSPHORUS IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT





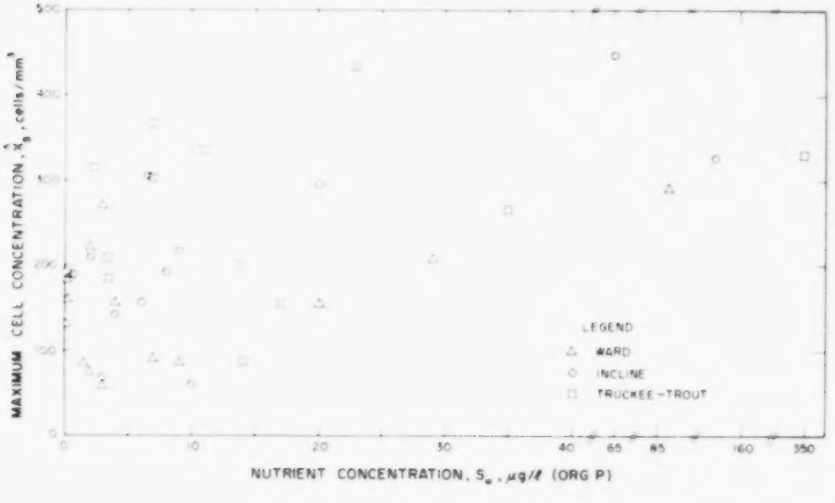


FIGURE C-6 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{bf}$, AND CELL CONCENTRATIONS, $\hat{X}_b$, WITH CONCENTRATION OF ORGANIC PHOSPHORUS IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT

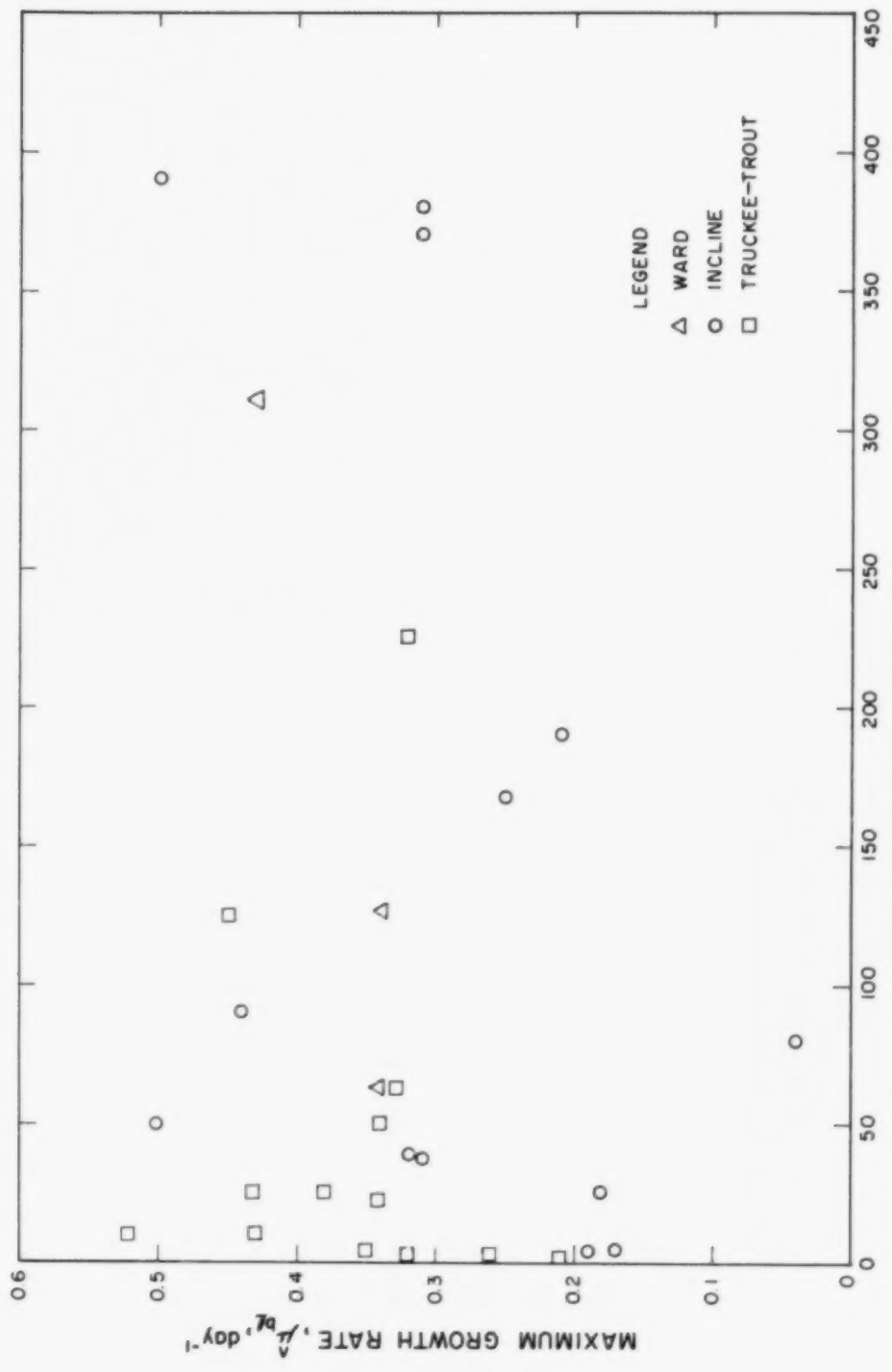
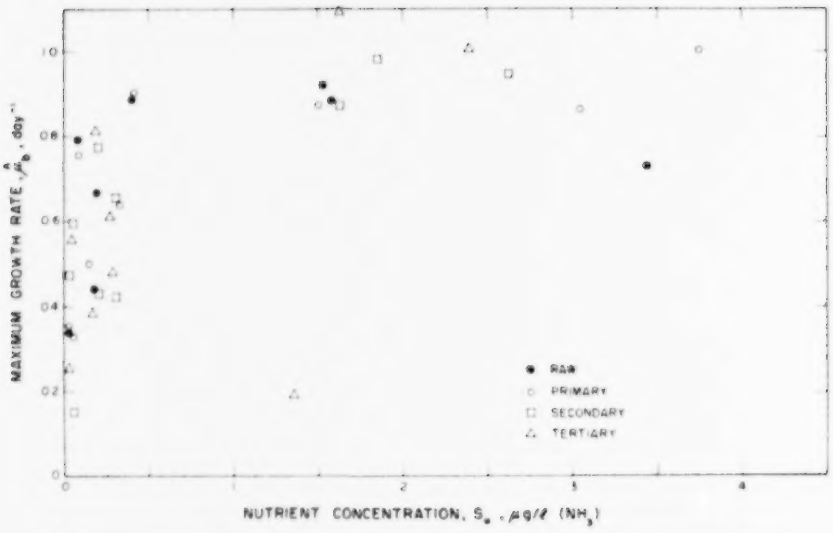
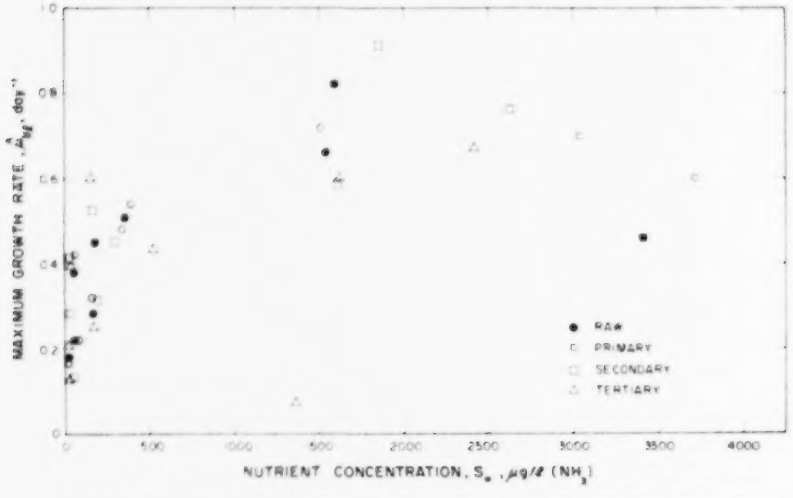


FIGURE C-7 VARIATION OF MAXIMUM GROWTH RATE, $\hat{\mu}_b$ ATTAINED AT THE END OF FIVE DAYS IN FLASK CULTURE, WITH CONCENTRATION OF IRON IN THE CULTURE FLASK USING VARIOUS CREEK WATER SAMPLES AS THE SOURCE OF NUTRIENT





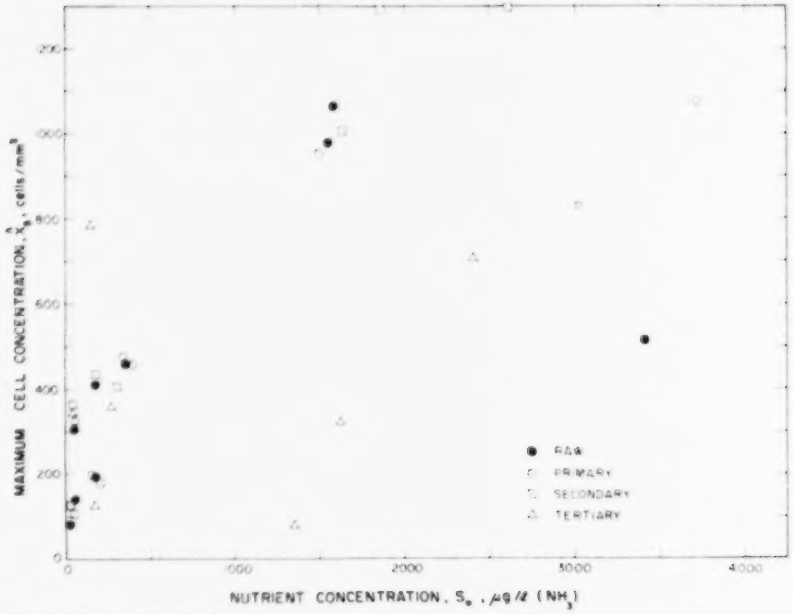
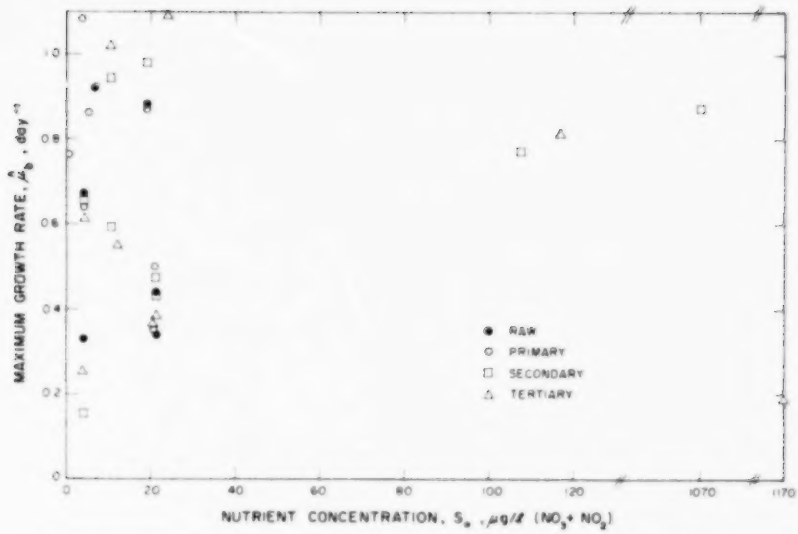
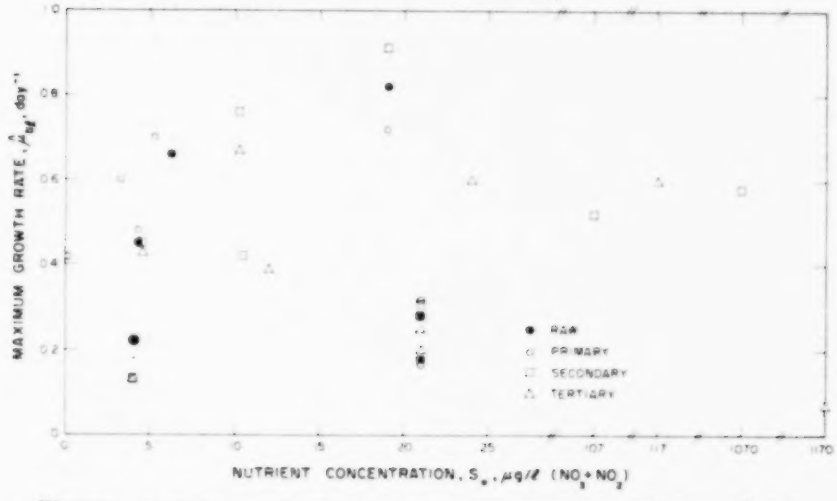


FIGURE C-8 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_o$ AND $\hat{\mu}_{o,\ell}$, AND CELL CONCENTRATIONS, $\hat{X}_o$, WITH CONCENTRATION OF AMMONIA NITROGEN IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT





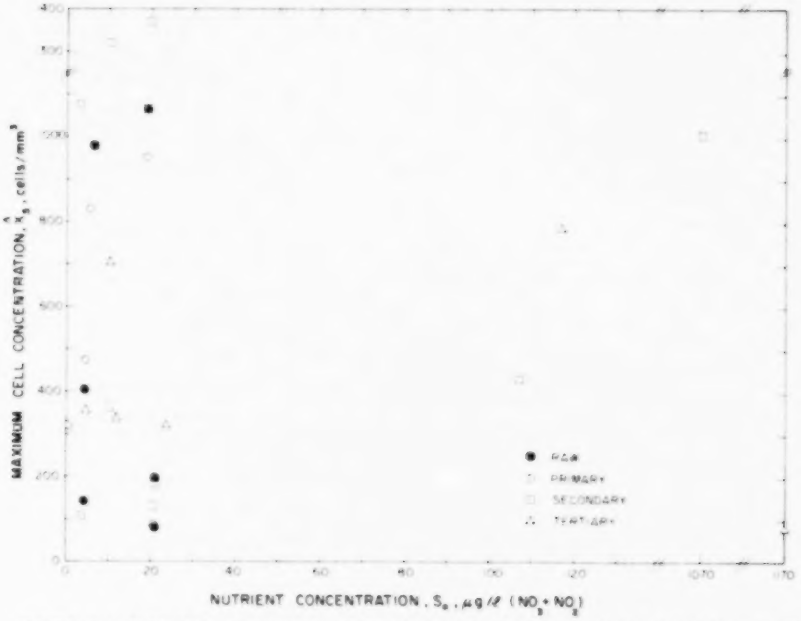


FIGURE C-9 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{b\ell}$, AND CELL CONCENTRATIONS, $\hat{X}_s$, WITH CONCENTRATION OF NITRATE AND NITRITE NITROGEN IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT

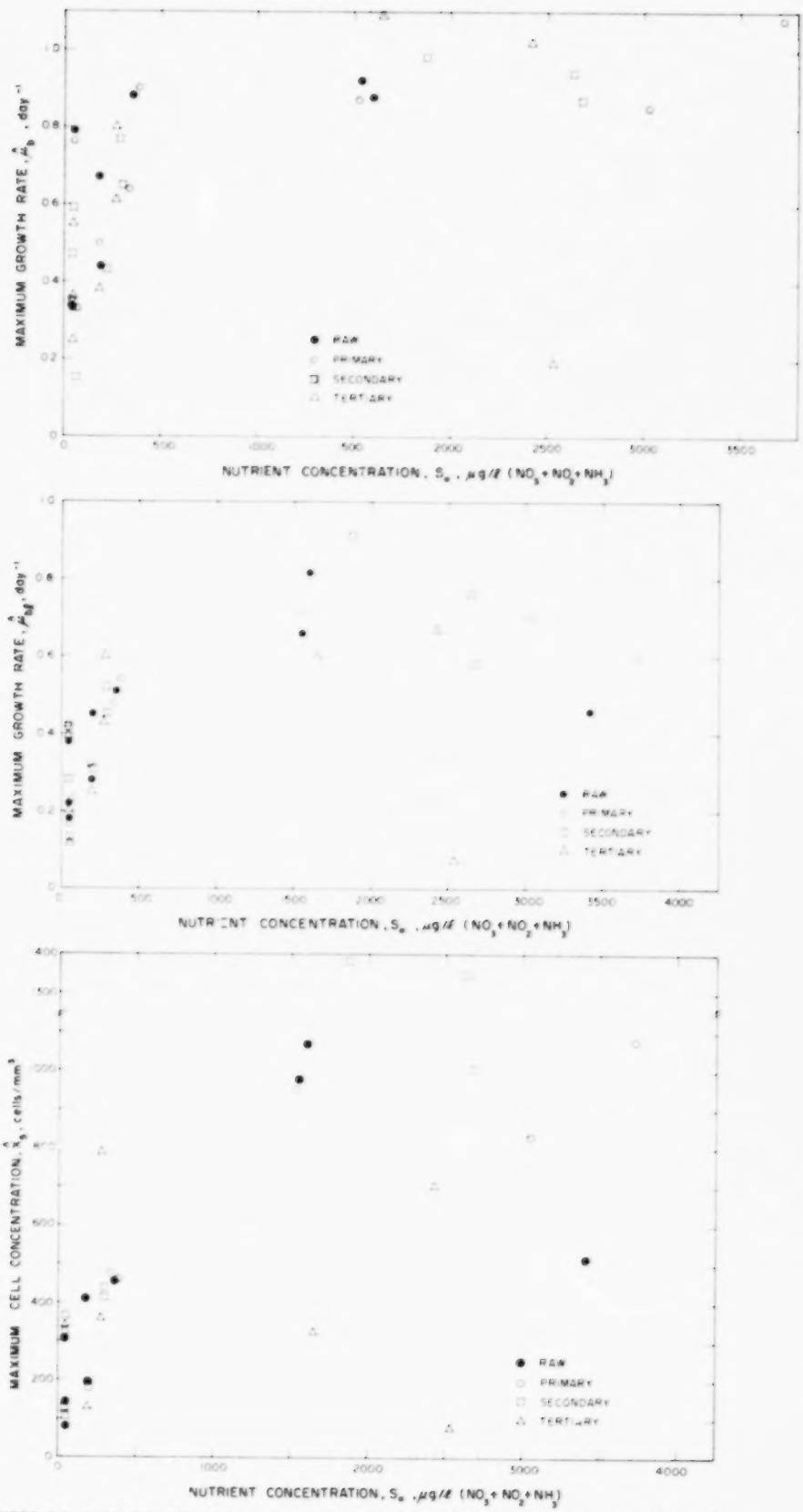
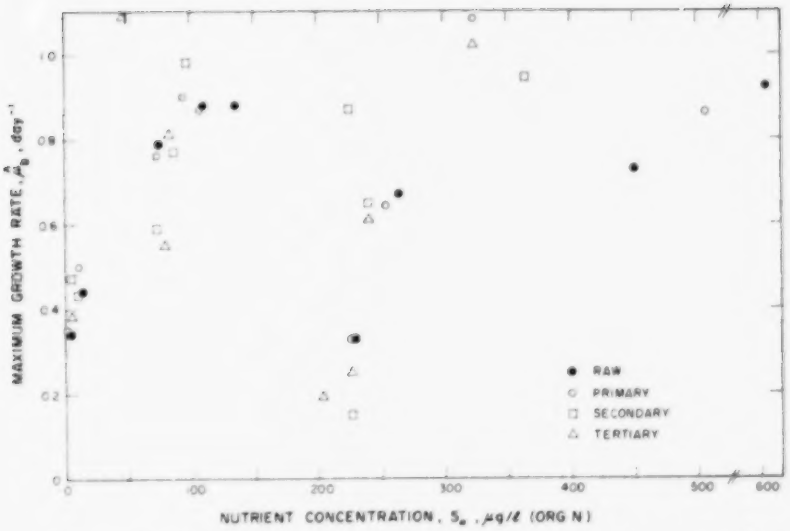
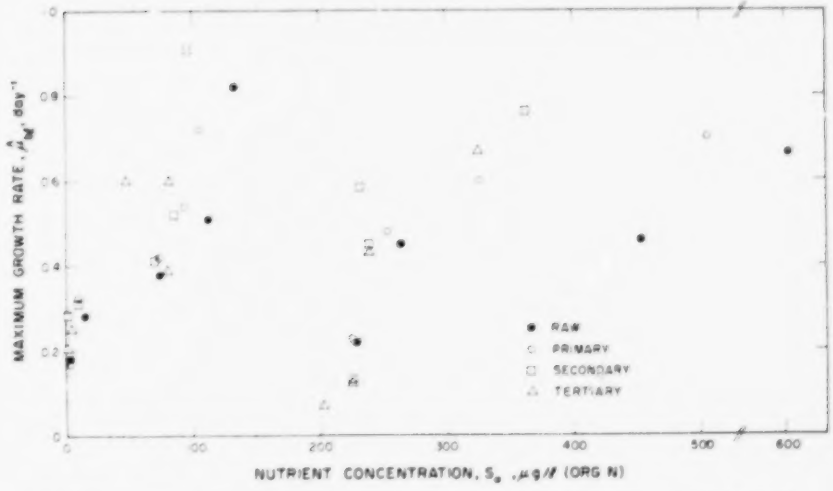


FIGURE C-10 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{bd}$, AND CELL CONCENTRATIONS, $\hat{N}_b$, WITH CONCENTRATION OF NITRATE, NITRITE, AND AMMONIA NITROGEN IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT





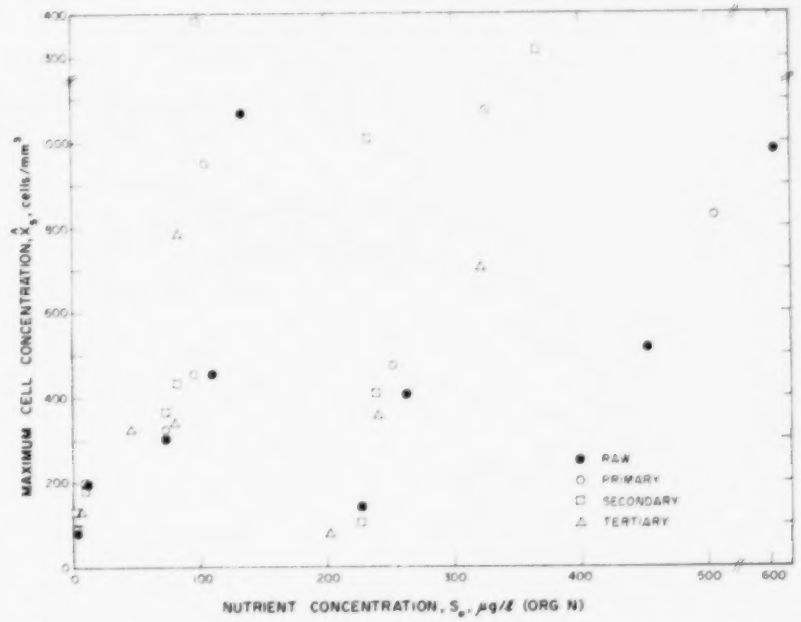


FIGURE C-11 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_n$ AND $\hat{\mu}_{net}$, AND CELL CONCENTRATIONS, $\hat{X}_s$, WITH CONCENTRATION OF ORGANIC NITROGEN IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT

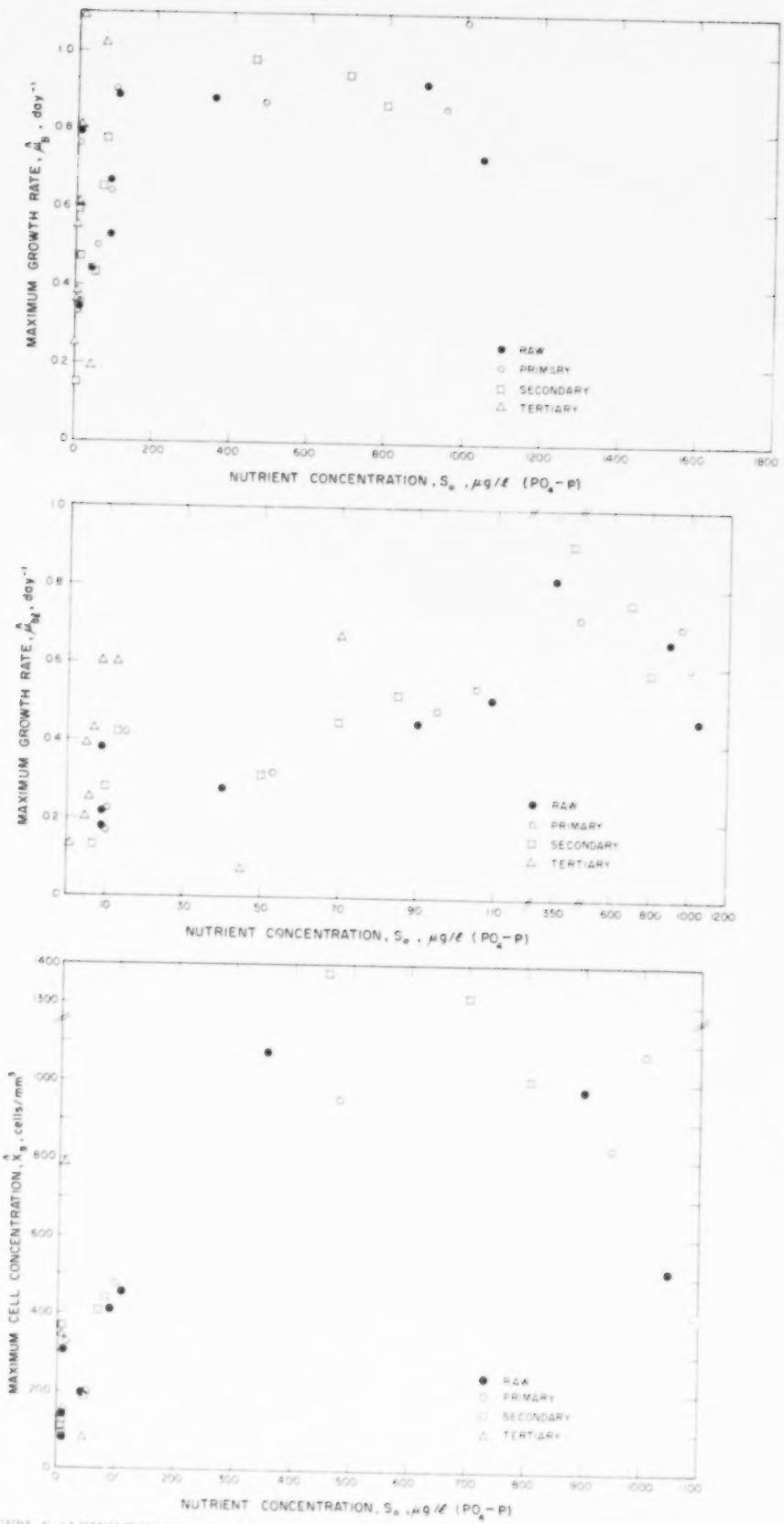
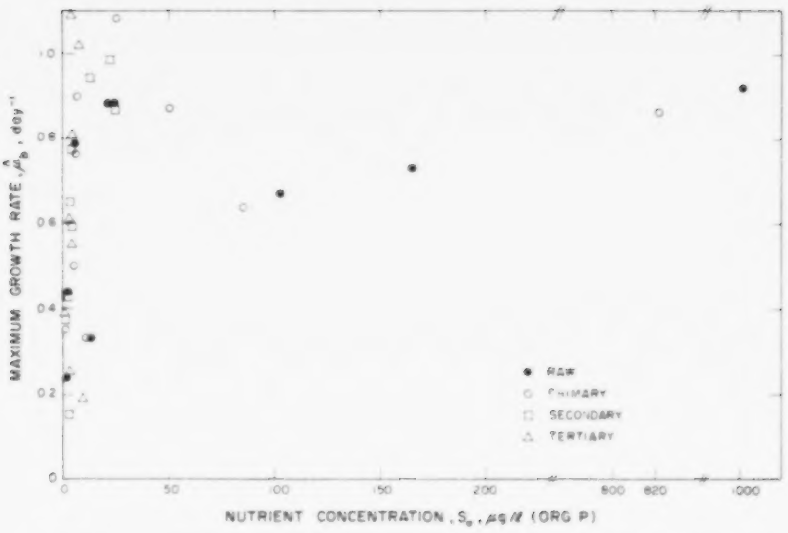
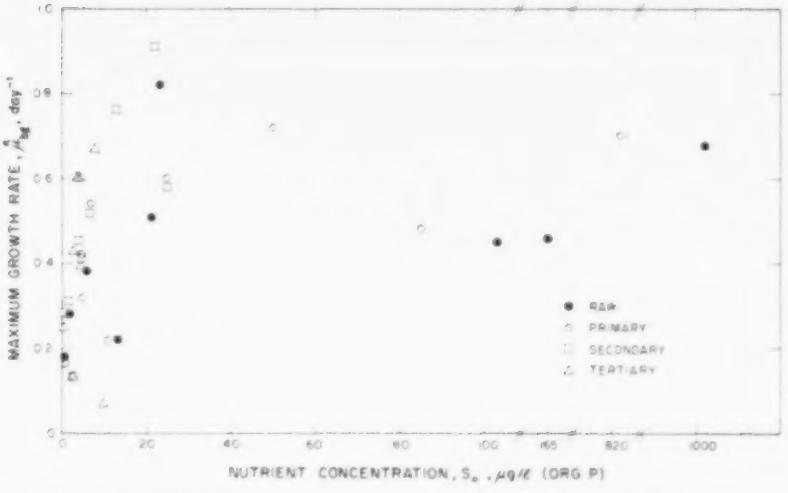


FIGURE C-12 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_B$ AND $\hat{\mu}_{Bf}$, AND CELL CONCENTRATIONS, $\hat{X}_B$, WITH CONCENTRATIONS OF PHOSPHATE AS PHOSPHORUS IN THE CULTURE PHASE FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT





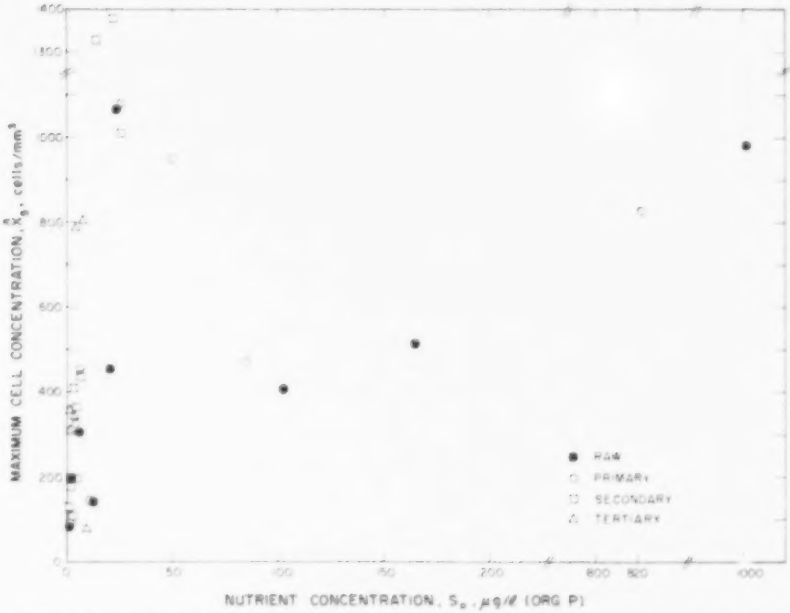


FIGURE C-13 VARIATION OF MAXIMUM GROWTH RATES, $\hat{\mu}_b$ AND $\hat{\mu}_{bf}$, AND CELL CONCENTRATIONS, $\hat{X}_b$, WITH CONCENTRATION OF ORGANIC PHOSPHORUS IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT

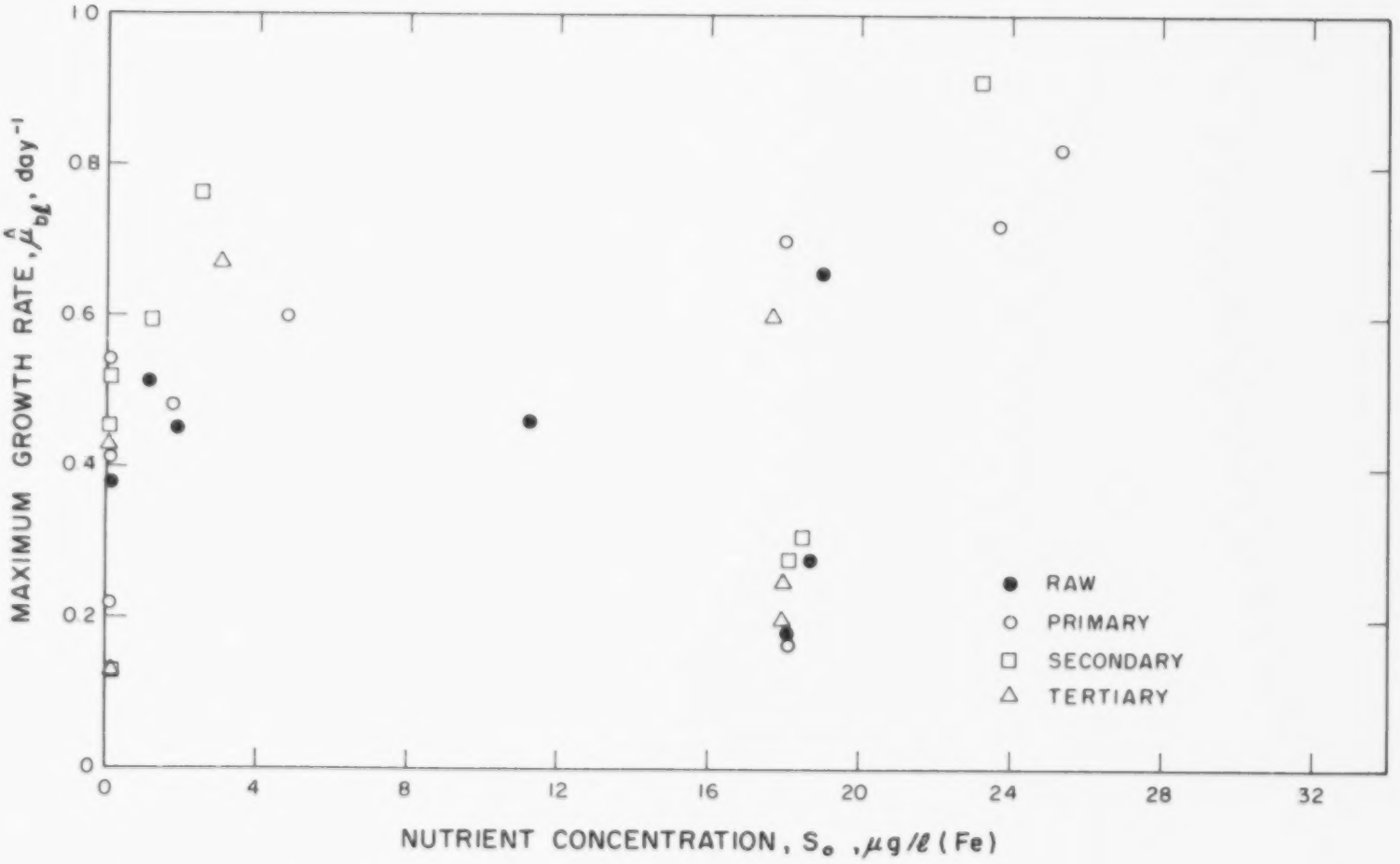
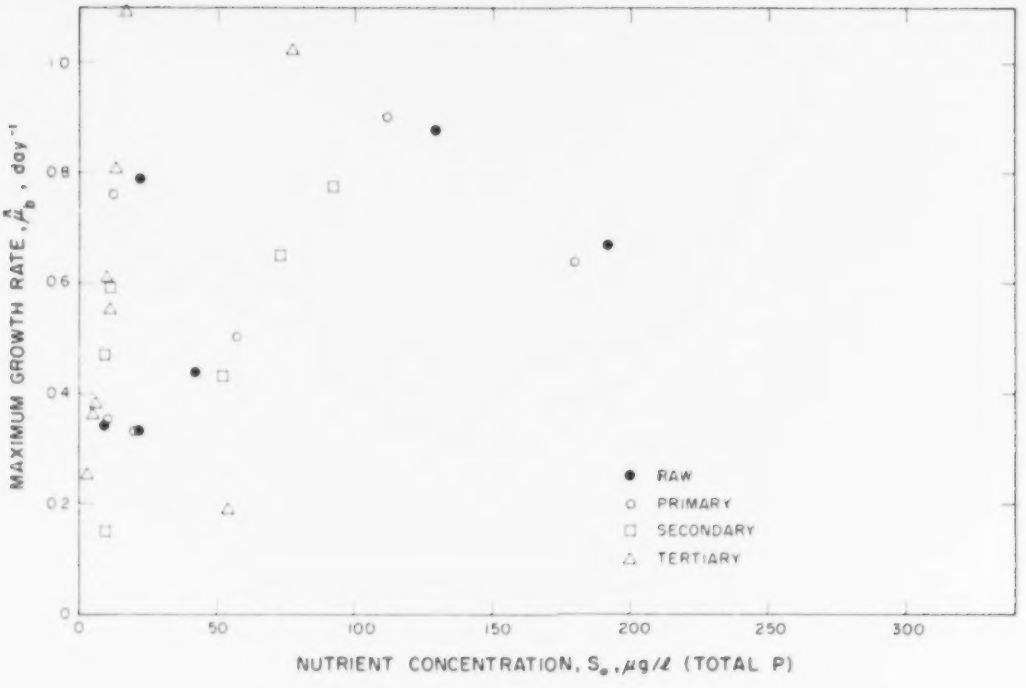


FIGURE C-14 VARIATION OF MAXIMUM GROWTH RATE, $\hat{\mu}_{b\ell}$, WITH CONCENTRATION OF IRON IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENT



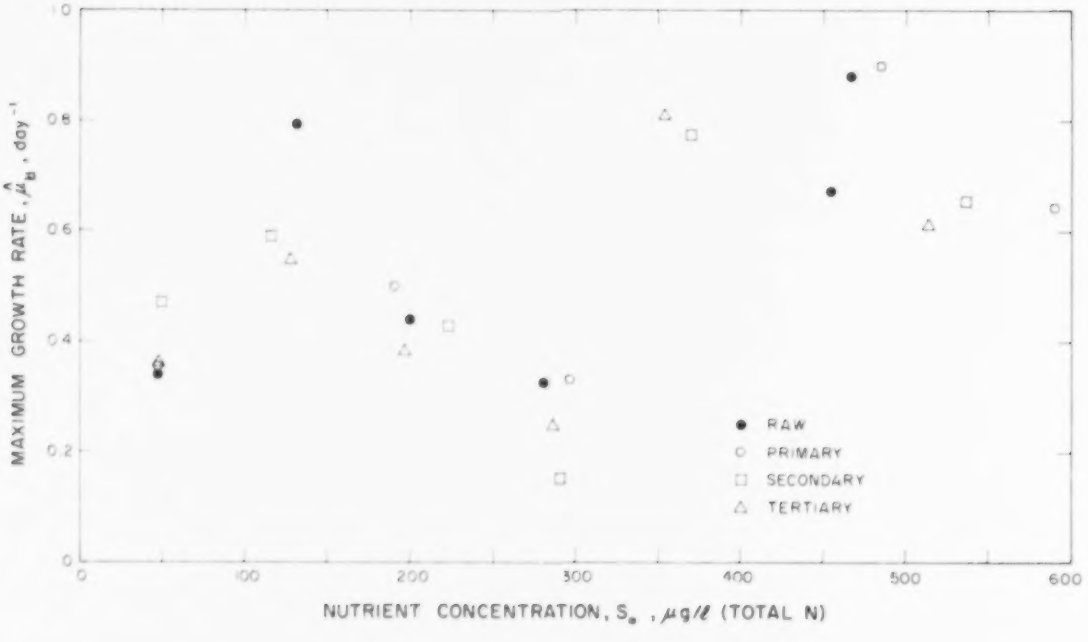


FIGURE C-15 VARIATION OF MAXIMUM GROWTH RATE, $\hat{\mu}_b$, WITH CONCENTRATION OF TOTAL NITROGEN AND TOTAL PHOSPHORUS IN THE CULTURE FLASK FOR WASTE WATER AND EFFLUENT SAMPLES FROM STPUD AS THE SOURCE OF NUTRIENTS

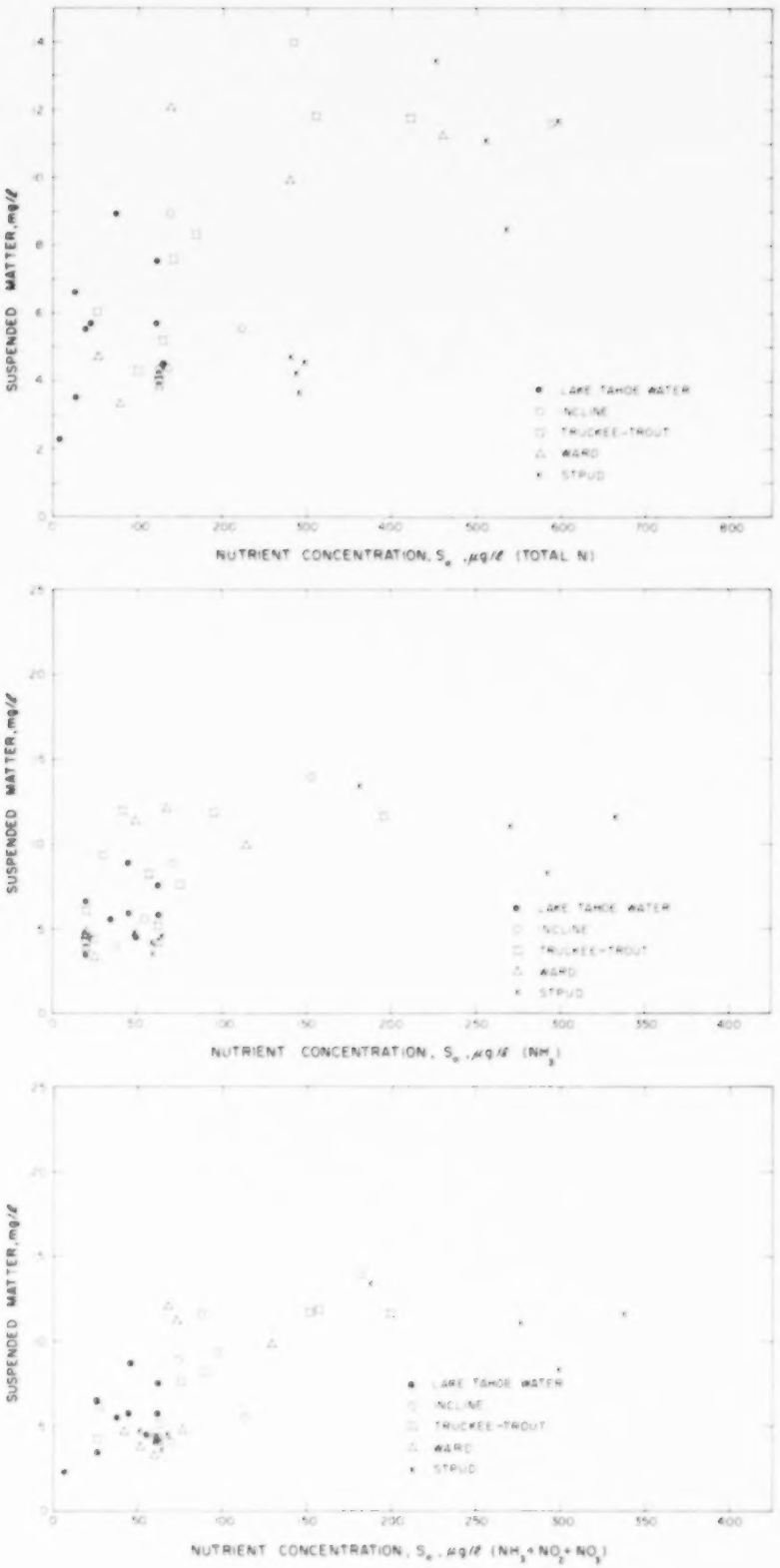
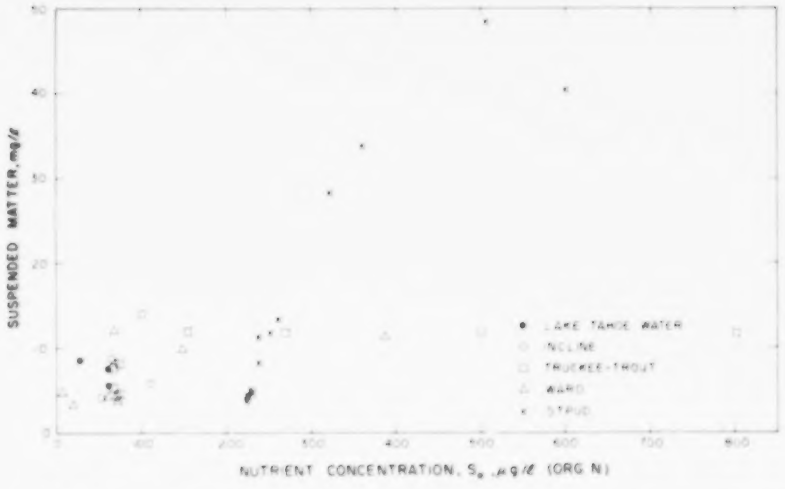
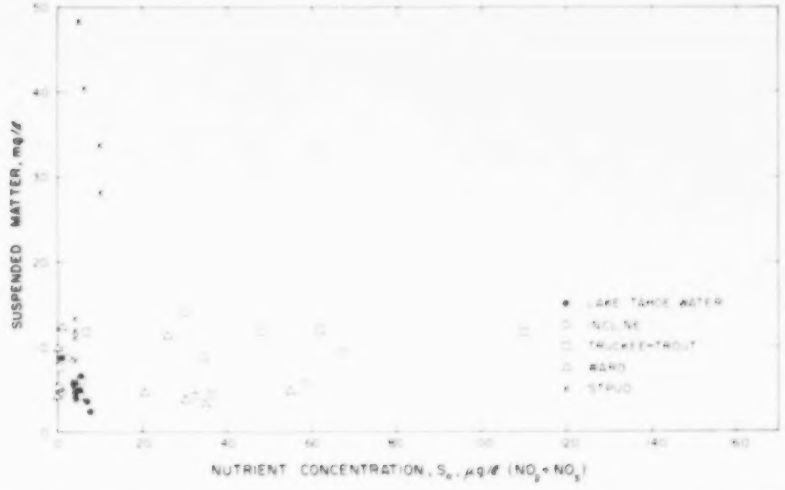


FIGURE C-9. VARIATION OF SUSPENDED MATTER WITH THE INITIAL CONCENTRATION OF VARIOUS FORMS OF NITROGEN IN THE CULTURE FLASK FOR VARIOUS WATER SAMPLES





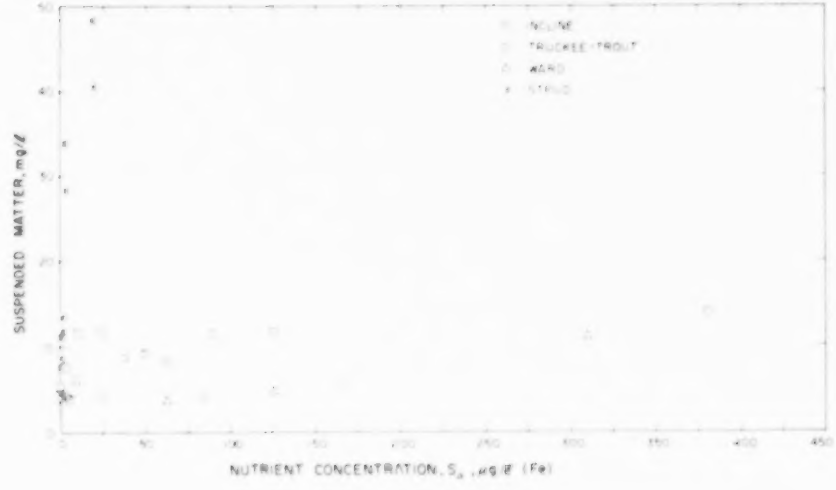
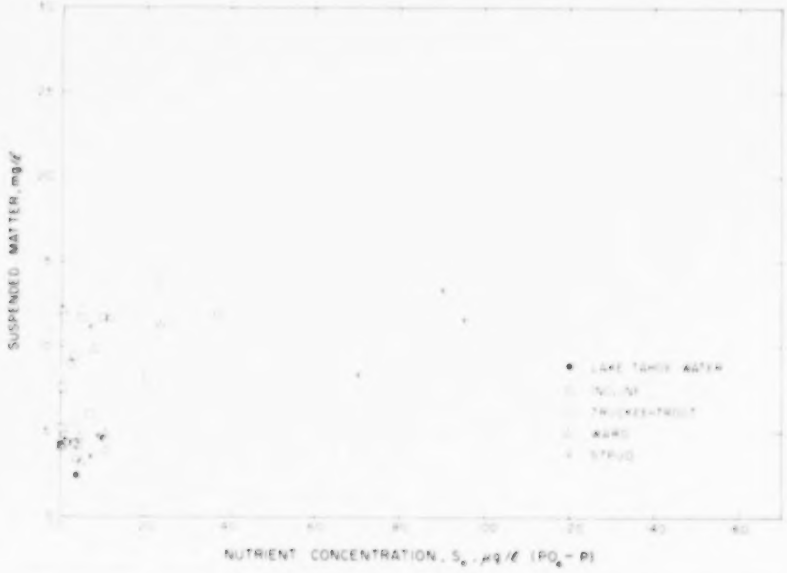
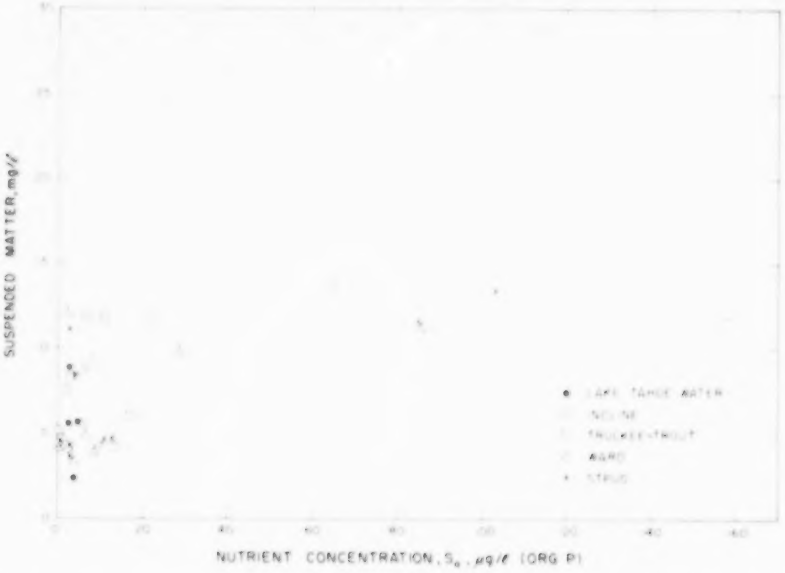


FIGURE C-17 VARIATION OF SUSPENDED MATTER WITH INITIAL CONCENTRATIONS OF NITROGEN AND OF REACTIVE IRON IN THE CULTURE FLASK FOR VARIOUS WATER SAMPLES.


SUSPENDED MATTER, mg/ℓ
NUTRIENT CONCENTRATION, S_o, µg/ℓ (TOTAL P)
LAKE TAHOE WATER
NCLIVE
TRUCKEE-TROUT
AGRO
STP.IO


SUSPENDED MATTER, mg/ℓ
NUTRIENT CONCENTRATION, S_o, µg/ℓ (PO_4-P)
LAKE TAHOE WATER
NCLIVE
TRUCKEE-TROUT
AGRO
STP.IO


SUSPENDED MATTER, mg/ℓ
NUTRIENT CONCENTRATION, S_o, µg/ℓ (ORG P)
LAKE TAHOE WATER
NCLIVE
TRUCKEE-TROUT
AGRO
STP.IO

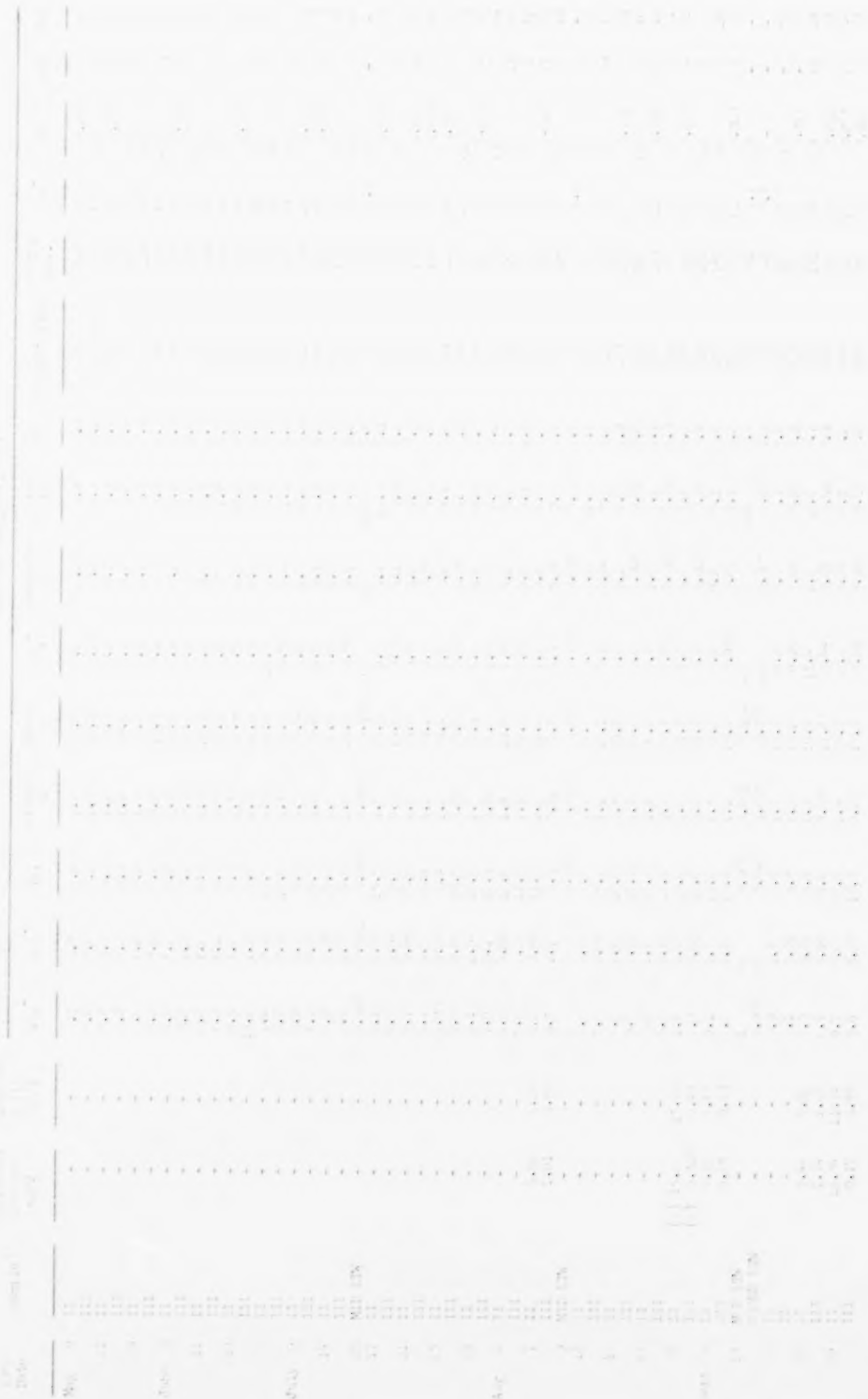
APPENDIX D

TABLE D-1. (Continued)

TABLE D-1 (Continued)

1968 Date	Array No. (1, myr)	Pond No.	Influent Description	Concentrations, mg/l NO_3-N	NH_4-N	Total N	PO_4-P	Total P	pH	Conductivity 10^{-6} mhos
Oct 11	7	1	LTW	5			<1			
	(5)	2	0.1 III	10			3			
	"	3	1.0 III	18			<1			
	"	4	1.0 III	15			<1			
	"	5	LTW	4			<1			
	"	6	0.1 II	4			<1			
	"	7	1.0 II	4			18			
	"	8	1.0 II	4			18			
Oct 14	"	1	LTW	4	78	144	3	12	7.8	85
	"	2	0.1 III	4	139	251	<1	4	7.9	91
	"	3	1.0 III	14	147	440	1	5	7.8	94
	"	4	1.0 III	11	190	414	<1	2	7.7	95
	"	5	LTW	4	140	268	<1	5	7.7	85
	"	6	0.1 II	1	85	201	<1	4	7.7	80
	"	7	1.0 II	<1	98	217	4	7	8.2	80
	"	8	1.0 II	<1	45	109	11	28	8.3	84
Oct 17	"	1	LTW		61		<1			
	"	2	0.1 III		120		<1			
	"	3	1.0 III		188		<1			
	"	4	1.0 III		238		<1			
	"	5	LTW		139		<1			
	"	6	0.1 II		98		<1			
	"	7	1.0 II		110		10			
	"	8	1.0 II		47		18			
Oct 21	"	1	LTW	5						
	"	2	0.1 III	4						
	"	3	1.0 III	10						
	"	4	1.0 III	7						
	"	5	LTW	3						
	"	6	0.1 II	3						
	"	7	1.0 II	4						
	"	8	1.0 II	2						
Oct 24	"	1	LTW	3	47		<1		7.3	96
	"	2	0.1 III	4	73		<1		7.6	90
	"	3	1.0 III	10	199		<1		7.7	98
	"	4	1.0 III	7	168		<1		7.6	91
	"	5	LTW	4	58		<1		7.6	94
	"	6	0.1 II	3	40		<1		7.6	88
	"	7	1.0 II	3	36		1		7.8	91
	"	8	1.0 II	4	42		4		8.0	91
Oct 28	"	1	LTW	12	41	156	<1	7		
	"	2	0.1 III	10	83	448	<1	<1		
	"	3	1.0 III	14	158	501	<1	4		
	"	4	1.0 III	12	183	463	<1	11		
	"	5	LTW	3	59	192	<1	4		
	"	6	0.1 II	3	57	254	<1	5		
	"	7	1.0 II	7	47	205	8	18	8.5	
	"	8	1.0 II	7	39	240	7	58	8.9	
Oct 31	"	1	LTW	22	25		<1			
	"	2	0.1 III	21	59		<1			
	"	3	1.0 III	25	259		<1			
	"	4	1.0 III	25	275		<1			
	"	5	LTW	17	52		<1			
	"	6	0.1 II	17	88		<1			
	"	7	1.0 II	15	15		<1			
	"	8	1.0 II	17	<5		5			
Nov 04	"	1	LTW	11	14		1		7.4	98
	"	2	0.1 III	10	18		<1		7.5	90
	"	3	1.0 III	11	275		4		7.5	95
	"	4	1.0 III	15	236		<1		7.5	95
	"	5	LTW	12	14		<1		7.5	88
	"	6	0.1 II	8	94		<1		7.5	89
	"	7	1.0 II	8	16		7		7.7	92
	"	8	1.0 II	11	8		4		7.9	91

APPENDIX E



APPENDIX F

APPENDIX C

APPENDIX H

TABLE I-1

CONCENTRATION OF CELLS IN PONDS

Date 1948	Influent Description	Pond No.	Cell Counts in Ponds, cells/mm³			
			Selenastrum gracile	Chlorella	Other Chlorophytes	Diatoms
Sept 15	1.0% II	1	0.02	2	1.5	0.3
[Army C,	0.1% III	2	1.2	1.1	-	0.2
θ = 10 days]	1.0% III	3	0.8	11	0.9	0.1
	1.0% III	4	2.6	3.7	0.1	0.1
	ITW	5	2.8	1.1	0.05	0.05
	0.1% II	6	1.5	4.3	-	0.4
	1.0% II	7	0.4	20	8.4	15.3
	1.0% II	8	1.2	20	4.2	1.0
Sept 16	1.0% II	1	0.2	3.8	4.5	0.5
	0.1% III	2	4.1	1.1	-	0.3
	1.0% III	3	1.0	3.7	1.0	0.5
	1.0% III	4	1.8	2.5	0.7	0.4
	ITW	5	0.9	3.5	0.1	0.5
	0.1% II	6	0.8	3.5	-	1.0
	1.0% II	7	0.5	12.0	5.0	16.0
	1.0% II	8	0.8	11.0	4.5	10.0
Sept 17	1.0% II	1	0.0	78	12.40	0.4
	0.1% III	2	0.2	4.5	0.2	0.1
	1.0% III	3	0.0	5	-	0.2
	1.0% III	4	0.3	40	-	0.05
	ITW	5	0.2	1.1	-	0.08
	0.1% II	6	0.2	74	0.2	1.56
	1.0% II	7	-	51	1	17
	1.0% II	8	-	47	8	0.7
Sept 24	1.0% II	1	-	95	4.2	1.0
	0.1% III	2	0.2	1.8	-	0.2
	1.0% III	3	2.1	15	4.3	0.4
	1.0% III	4	0.2	20	-	0.1
	ITW	5	0.1	0.2	-	0.4
	0.1% II	6	2.8	41	-	2
	1.0% II	7	-	23	5.2	27
	1.0% II	8	-	99	1.8	11
Sept 25	1.0% II	1	-	58	4.6	1.3
	0.1% III	2	0.4	1.2	-	0.2
	1.0% III	3	-	19	-	0.4
	1.0% III	4	1.0	1.8	-	1.05
	ITW	5	-	0.2	-	0.8
	0.1% II	6	-	44	-	3.7
	1.0% II	7	-	20	0.38	22
	1.0% II	8	-	88	0.1	12.3
Sept 27	1.0% II	1	-	50	7.2	7.4
	0.1% III	2	0.2	23	-	5.7
	1.0% III	3	-	18	-	0.4
	1.0% III	4	-	14	-	0.8
	ITW	5	-	0.9	-	0.4
	0.1% II	6	-	40	-	5.8
	1.0% II	7	-	19	0.9	15.3
	1.0% II	8	-	42	-	14.1

TABLE I-1 (Continued)

Date 1968	Influent Description	Pond No.	Cell Counts in Ponds, cells/mm^3			
			Selenastrum gracile	Chlorella	Other Chlorophytes	Diatoms
Sept 30	1.0% II	1	-	17	4.2	2.2
	0.1% III	2	-	1.1	-	0.3
	1.0% III	3	-	26	-	0.1
	1.0% III	4	-	16	-	0.7
	LTW	5	-	2.5	-	1.1
	0.1% II	6	-	30	-	2.13
	1.0% II	7	-	31	-	22
	1.0% II	8	-	44	-	8.6
Oct 2	1.0% II	1	-	37	6	3.5
	0.1% III	2	-	5.9	-	0.2
	1.0% III	3	-	24	-	0.7
	1.0% III	4	-	19	0.7	0.4
	LTW	5	-	3.6	-	2.8
	0.1% II	6	-	24.8	-	1.1
	1.0% II	7	-	38	-	21
End of Assay 6	1.0% II	8	-	28	-	10
Oct 9 [Assay 7, θ = 5 days]	LTW	1	-	2.8	0.07	0.08
	0.1% III	2	-	3.3	-	0.2
	1.0% III	3	-	12.6	-	0.2
	1.0% III	4	-	11.4	-	0.2
	LTW	5	-	0.2	-	0.1
	0.1% II	6	-	16	-	0.1
	1.0% II	7	0.7	18.4	0.9	10
	1.0% II	8	0.6	16	-	8
Oct 11	LTW	1	-	2.2	-	0.2
	0.1% III	2	-	2.0	-	0.2
	1.0% III	3	-	7.7	-	0.2
	1.0% III	4	-	5.7	0.2	0.3
	LTW	5	-	0.8	-	0.1
	0.1% II	6	-	5.9	-	0.7
	1.0% II	7	1.0	22	0.2	10
	1.0% II	8	0.7	45	-	7
Oct 15	LTW	1	-	1.0	-	0.6
	0.1% III	2	-	3.1	-	0.1
	1.0% III	3	-	5.6	-	0.5
	1.0% III	4	-	3.0	-	0.8
	LTW	5	-	1.9	-	0.3
	0.1% II	6	-	5.4	-	2.0
	1.0% II	7	-	25	-	8.6
	1.0% II	8	1.3	24	-	9.6
Oct 17	LTW	1	-	3.2	-	0.8
	0.1% III	2	-	4.6	-	0.5
	1.0% III	3	-	4.8	-	0.4
	1.0% III	4	-	3.0	-	0.3
	LTW	5	-	2.0	-	0.2
	0.1% II	6	-	9.6	-	0.6
	1.0% II	7	0.6	22	-	3.4
	1.0% II	8	0.5	36	-	8.5

TABLE I-1 (Continued)

Date 1968	Influent Description	Pond No.	Cell Counts in Ponds, cells/mm^3			
			Selenastrum gracile	Chlorella	Other Chlorophytes	Diatoms
Oct 21	LTW	1	-	3.4	-	0.4
	0.1% III	2	-	3.2	-	1.1
	1.0% III	3	-	3.3	-	0.1
	1.0% III	4	-	3.2	-	0.3
	LTW	5	-	2.2	-	0.3
	0.1% II	6	-	7.1	-	1.6
	1.0% II	7	1.2	22	1.2	13.3
	1.0% II	8	0.6	20	0.6	20
Oct 23	LTW	1	-	2.3	-	0.4
	0.1% III	2	-	2.5	-	1.5
	1.0% III	3	-	2.9	-	0.4
	1.0% III	4	-	3.2	-	0.3
	LTW	5	-	1.0	-	0.3
	0.1% II	6	-	3.5	-	0.8
	1.0% II	7	1.2	25	-	10
	1.0% II	8	1.2	23	-	16
Oct 25	LTW	1	-	1.3	-	0.7
	0.1% III	2	-	1.7	-	2.2
	1.0% III	3	-	3.1	-	0.2
	1.0% III	4	-	2.2	-	0.1
	LTW	5	-	1.4	-	0.4
	0.1% II	6	-	8.0	-	1.7
	1.0% II	7	2.3	70	-	22
	1.0% II	8	3.3	60	1.4	46
Oct 28	LTW	1	-	3.6	-	0.4
	0.1% III	2	-	1.5	-	1.6
	1.0% III	3	-	4.3	0.1	0.5
	1.0% III	4	-	4.6	-	0.2
	LTW	5	-	0.5	-	0.4
	0.1% II	6	-	11.1	-	1.6
	1.0% II	7	-	10	-	22
	1.0% II	8	1.8	20	0.6	25
Oct 30	LTW	1	-	1.5	-	1.4
	0.1% III	2	-	-	-	10.3
	1.0% III	3	-	2.9	0.7	1.6
Debris more than usual	1.0% III	4	-	3.7	0.2	2.6
	LTW	5	-	-	0.1	0.4
	0.1% II	6	-	4.9	-	2.2
	1.0% II	7	1.2	85	-	22
	1.0% II	8	1.7	31	1.6	39
Nov 1	LTW	1	-	-	-	0.6
	0.1% III	2	-	-	-	5.2
	1.0% III	3	-	3.7	-	0.8
	1.0% III	4	-	4.6	-	0.2
	LTW	5	-	-	-	0.3
	0.1% II	6	-	2.0	-	1.1
	1.0% II	7	2.0	83	0.8	23
	1.0% II	8	1.2	17	0.6	34
End of Assay 7						

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